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PROTEIN FREE FILTRATE OR DIALYSATE SOME EXPERIENCES WITH AUTOMATION IN A CLINICAL CHEMISTRY LABORATORY, WITH SPECIAL REFERENCE TO THE ROUTINE BLOOD GLUCOSE DETERMINATIONS*

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In 1957 two papers, one by Skeggs[16] and the other by Marsh, et al.,[13] were published, describing a new automatic dialyzing machine, the Auto-Analyzer,** capable of producing accurate and reproducible blood urea nitrogen tests automatically. This machine once set up would perform the tests without further manual attention, thus freeing the technologist for other determinations. It was mentioned that this equipment could be used satisfactorily to produce, in a similar way, results of as many as 40 blood glucose determinations in one hour.

The purpose of this paper is to describe the AutoAnalyzer and present a blood glucose technic. The precision of the AutoAnalyzer was compared with the precision of a modified Folin and Wu technic and a modified Somogyi technic. Normal fasting blood glucose values were determined using the AutoAnalyzer.

METHODS

The AutoAnalyzer. As shown in plate 1, the AutoAnalyzer consists of six units or "Modules." Module A is the sampling device and consists of a plate made to hold 40 small plastic cups. A small arm situated to the left holds the sampling tube which dips in and out of the cups, aspirating predetermined volumes of fluid into the apparatus. During each cycle the sample is aspirated for two-thirds of the time and air is aspirated for the one-third of the time. The speed of transport of the sample cups can be adjusted so that 20, 40, or 60 samples may pass under the sampling arm per hour.

The Module B is the proportioning pump. This consists of a pump, which is a series of steel rollers mounted between two chains, the chains being driven by sprockets connected to the motor by means of a clutch. These rollers traverse a rocking platen upon which rests a manifold.

The manifold consists of a number of precision made tygon plastic tubes. The internal diameter of these tubes is held to extremely close tolerances during manufacture so that specific volumes of fluids or air may be delivered at given rates per unit of time. Since all manifold tubes

* 1st Registry Award 1958, 1st ASMT Award 1958, 2nd SPF Educational or Procedural Award 1958. Read before the 26th Annual ASMT Convention, Milwaukee, Wisconsin, June 1958.
** The AutoAnalyzer is manufactured by the Technicon Instruments Corporation, Chauncey, New York.

are compressed at the same time by the proportioning pump, volumes of samples and reagents remain constant throughout the equipment at all times. Each sample is divided into many small segments by the injection of air bubbles into the streams of fluid at regular intervals. The air serves a two-fold purpose. First, it separates samples and prevents mixing of two adjacent tests. Second, the air has a cleaning action on the plastic tubing, preventing contamination.

The manifold is connected through mixing coils to the Module C which consists of a dialyzing chamber. The dialyzing chamber is manufactured of acrylic plastic and is made in two parts. The upper and lower plates of the dialyzing chamber are mirror images of each other and when clamped together have a spiral tube 87 inches long by 1/16 inch in diameter running through them. These two sections are separated in use by a tightly stretched cellophane membrane, giving an extremely efficient dialyzing chamber. The two acrylic plastic plates are clamped together first by two large stainless steel discs to prevent warping of the plastic plates, followed by a steel washer and a clamping nut.

The cellophane membrane should be changed once per week or more often if drying out or clogging of the pores in the membrane causes large changes in the slope of the calibration curve.

The sample mixed with diluent enters through the top half of the dialyzing plate, each sample being separated from its neighbor by air and saline. The waste, after dialysis, is removed through a tygon tube to the drain. The recipient fluid enters through the bottom half and passes through the spiral channel, picking up diffusible materials from the diluted blood sample. Further reagents are added after dialysis and the mixture passed through a mixing coil to the constant temperature heating bath. This is labeled D in Plate 1.

Over 400 inches of 1/8 inch diameter glass tubing is coiled and submerged in diethylene glycol. The temperature is held at 95 C. plus or minus 0.1 C. by a mercury thermostat. To ensure uniform temperature distribution throughout the bath a mechanical stirrer is incorporated. Each specimen is heated in this bath for approximately five minutes at a flow rate of 40 samples per hour.

From the heating bath the reactants are fed into a glass flow cuvette having a light path of 6 mm., in a dual beam null balance photoelectric colorimeter. This colorimeter (Module E) uses interference filters (having a half band of 10 millimicrons and a peak transmittance of 35 per cent) to provide monochromatic light. Changes in chromogen content of the reactants in the flow cell cause changes in electrical potential to be produced between the two balanced photo cells. These changes are recorded as a series of peaks showing transmittance per cent on the moving chart paper of the ratio-recording apparatus marked F in Plate 1.

PRINCIPLE OF THE AUTOANALYZER TECHNIC

The AutoAnalyzer utilizes the method of dialysis to separate the glucose from the protein in blood. Since the blood sample is diluted with 0.9 per cent NaCl no hemolysis of red cells takes place, hence the presence of non-glucose reducing substances is held to a minimum and a true glucose level is given.

The colorimetric procedure used is similar to that described by Hoff-

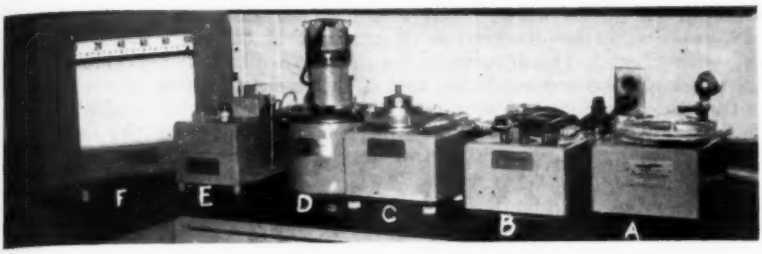


Plate I. The AutoAnalyzer.

A. Sampling Device. B. Proportioning Pump. C. Dialyzer. D. Constant Temperature Bath. E. Photoelectric Colorimeter. F. Ratio-Recording Apparatus.

man[8] and is based on the reduction of yellow potassium ferricyanide in alkaline solution, to colorless potassium ferrocyanide in proportion to the concentration of glucose present. Alkaline potassium cyanide is added as a reaction sensitizer.

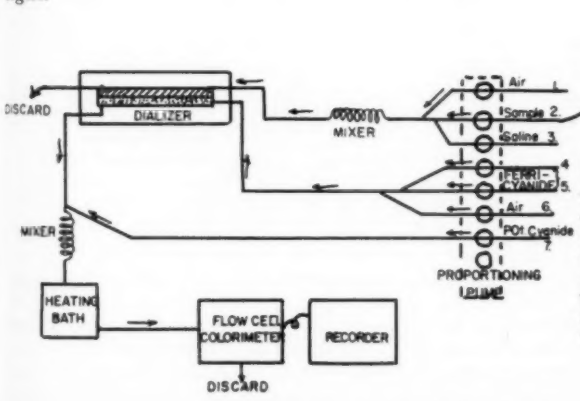
Technic for the Determination of Blood Glucose by the AutoAnalyzer

Reagent

All reagents are prepared according to the formulae suggested in the AutoAnalyzer Manual[5] published by the Technicon Company.

Technic

The AutoAnalyzer was operated in accordance with the manufacturer's instructions using a modified manifold constructed as shown in Figure 1. Interference filters peaking at 420 mu provided monochromatic light.



KEY: Manifold Tube Sizes

No.	I.D.	Approx vol. per min.
1.	0·056"	1·00 ml.
2.	0·035"	0·42 ml.
3.	0·073"	2·00 ml.
4.	0·081"	2·50 ml.
5.	0·035"	0·42 ml.
6.	0·040"	0·52 ml.
7.	0·065"	1·50 ml.

Figure 1. Schematic drawing of the manifold.

About 0.5 ml. of whole blood was used for the test.

A representative illustration of a series of blood sugar analyses is shown in Fig. 2. The transmittance per cent of the standards is plotted on the logarithmic coordinate and the concentration is plotted on the arithmetic coordinate of semilogarithmic graph paper. A curved plot as shown in Fig. 3 is formed. The transmittance per cent of the unknowns can now be read from this graph and the glucose results expressed directly in milligrams per cent.

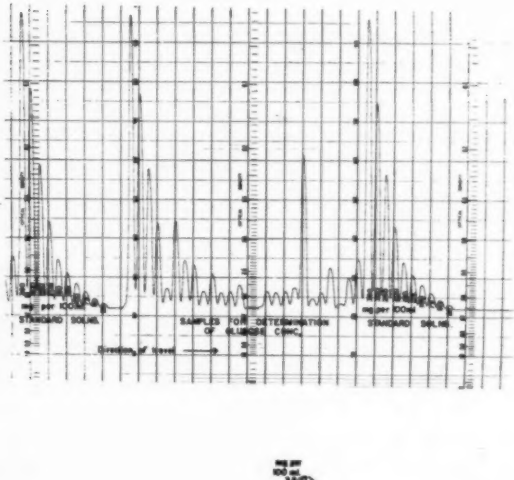


Figure 2. A representative illustration of the peaks recorded during a routine series of blood glucose determination.

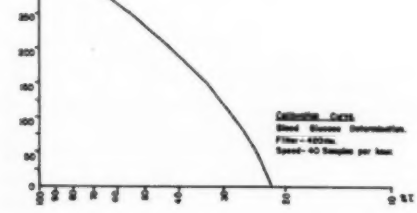


Figure 3. A typical calibration curve obtained from the analysis of standard glucose solutions.

Routine Laboratory Method of Deproteinization and Blood Glucose Determination

Protein free filtrates were prepared using Haden's modification of the Folin and Wu tungstic acid method (Hawk et al., 1954).[7]

The total reducing substances present in the filtrate were determined by Fiorentino's modification[4] of Folin and Wu's alkaline copper tartarate phosphomolybdic acid technic.

Somogyi Deproteinization Method

The deproteinization method used was Somogyi's Zinc Sulphate, Sodium Hydroxide Method[9] (Hoffman, 1941). The glucose contained in the protein free filtrate was determined by the same copper reduction method given above.

EXPERIMENTAL

Reproducibility of AutoAnalyzer, Laboratory Method and Macro Somogyi Method

All pools of oxalated blood used in the following experiments were treated with sodium fluoride in the proportion of 200 mg. per 100 ml. to preserve the glucose levels (Mackie, 1945).[12]

Known weights of dry analytical grade dextrose were added to three 100 ml. portions of a pool of blood so that the final concentration of glucose in each portion was approximately 50, 150 and 200 mg. per 100 ml.

Twenty determinations were made on each glucose concentration by the three methods and the results were recorded. The samples examined by the Folin and Wu method were included with the regular daily run of routine blood glucose determinations from the hospital. One technologist analyzed those done by the Somogyi method.

In spite of the apparently high reproducibility of the AutoAnalyzer as demonstrated by the results of the previous experiment, it was found that the calibration curve was not exactly reproducible from run to run. It was necessary, therefore, to show that this change affects the samples in each run as well as the curve.

In order to do this 11 blood samples of varying glucose content from 68 mg. per 100 ml. to 285 mg. per 100 ml. were obtained from hospital patients. In order to preserve the glucose levels, the 10 ml. samples of blood were drawn into Vacutainers* containing 20 mg. potassium oxalate and 25 mg. sodium flouride as the anticoagulant. The samples were analyzed by the AutoAnalyzer on two consecutive days.

Precision Recovery of the Three Methods

To examine the precision of recovery of the three methods amounts of 5 mg., 16 mg., 26 mg., and 32 mg. of anhydrous dextrose were weighed into each of four 25 ml. volumetric flasks and pooled blood added to volume. The contents of the flasks were well mixed and analyzed in duplicate by the three methods. The samples were placed on the sample plate of the AutoAnalyzer in order of ascending glucose value.

The previously determined glucose content of the pool was subtracted from the total glucose found by each method and the difference recorded as the amount recovered in terms of mg. per 100 ml.

Since the AutoAnalyzer is a mechanical device, it was thought that some contamination may be carried from specimen to specimen, especially where a high glucose precedes a low one. To check this, amounts of 8.8 mg., 18.2 mg., 30.8 mg., 46.6 mg., and 60.1 mg. of anhydrous dextrose were weighed into five 25 ml. volumetric flasks and each made up to volume with pooled blood having a glucose content of 50 mg. per 100 ml., as determined by analyzing replicate samples of the pool by the AutoAnalyzer. The contents of the flasks were well mixed. The flasks were numbered 1 to 5 and the pool was number 6. Using a table of

*Vacutainers are manufactured by Becton, Dickinson and Company, Rutherford, New Jersey.

random numbers (Dixon and Massey, 1951)[3] for selection, the samples were processed in random order to simulate a routine run of blood glucose determinations in which high, low and intermediate glucose concentrations follow each other in unknown order. The samples were analyzed as decribed in the section on Methods.

The value of the glucose content of the pool was subtracted from the total and the remainder recorded in terms of mg. per 100 ml. of glucose recovered.

Calculation of Normal Values by AutoAnalyzer

After a 12 hour fasting period, 10 ml. of blood was obtained from each of 48 healthy males and females between the ages of 19 and 50 years. To preserve the glucose levels, the blood samples were drawn into Vacutainers containing 20 mg. potassium oxalate and 25 mg. sodium fluoride. The blood and anticoagulant were well mixed by turning the tubes end over end at least 25 times.

The 48 samples of blood were analyzed in duplicate by the AutoAnalyzer.

RESULTS

Reproducibility Experiment

The standard deviation and 95 percentile range of the three glucose methods were calculated. These results are shown in Table I.

TABLE I

Method	Number of Samples	Variance S^2	Standard Deviation S.D.	95 Percentile Range
Routine Lab. Method	60	15.47	3.94 mg./100 ml.	±7.88 mg./100 ml.
Somogyi	60	3.6	1.9 mg./100 ml.	±3.80 mg./100 ml.
AutoAnalyzer	60	1.32	1.15 mg./100 ml.	±2.25 mg./100 ml.

Comparison of reproducibility of the three blood glucose methods.

As shown in Table II, the reproducibility of the AutoAnalyzer from day to day in spite of the slight changes in the slope of the calibration curve, is amply demonstrated by the close correspondence of the results of the 11 samples analyzed on two consecutive days.

TABLE II

# Samples	mg./100 ml. Change
5	1
3	2
2	3
1	4

Standard Deviation for the Method = ± 2.25 mg./100 ml.
Results of blood glucose determinations of 11 samples taken from hospital patients and analyzed on two consecutive days.

Recovery Experiments

Table III shows the values of the glucose recovered by all three methods. It will be seen that the precision of the AutoAnalyzer in the recovery of even very small quantities is excellent. Table IV shows the excellent recovery obtained by the AutoAnalyzer under conditions of routine use where blood glucose samples of widely differing value may

TABLE III

	AutoAnalyzer		Laboratory		Somogyi	
Pool Blood Value	100 mg./100 ml.		125 mg./100 ml.		94 mg./100 ml.	
Glucose added mg./100 ml.	Amount Recovered	Per cent	Amount Recovered	Per cent	Amount Recovered	Per cent
20	21	105	20	100	20	100
20	21	105	12	57	18	90
64	63	98.5	68	106	69	106
64	65	101.5	52	81.5	66	103
104	104	100.0	106	102	102	98
104	106	102.0	100	96	108	104
128	129	101.5	142	111	138	108
128	130	100.5	142	111	134	104.5

Comparison of recoveries by the three glucose methods.

be found adjacent to each other in a single run.

Normal Values for the AutoAnalyzer Technic

Statistical analysis of the 48 normal fasting blood glucose determinations showed the mean value to be 78 mg. per 100 ml. and the standard deviation 5.53 mg. per 100 ml. To establish the 99 percentile limits of the normal range three times the standard deviation was used. This gave limiting values of 61 to 95 mg. per 100 ml.

TABLE IV

Sample #	1	5	4	2	3	3	1
Total Glucose Found, mg./100 ml.	85	290	237	123	172	172	85
Amount Recovered in mg./100 ml.	35	240	187	73	122	122	35
Percent Recovered	99.5	99.5	100.2	100.5	99	99	99.5

Representative results of the recovery achieved by the AutoAnalyzer in a simulated run of blood glucose determinations. The pool had a glucose content of 50 mg. per 100 ml.

DISCUSSION

The shortcomings of the various methods in common use in the laboratory for the determination of blood glucose have been pointed out many times by many authors. The modifications made to improve these methods are innumerable. The Symposium on Blood Glucose by Sunderman et al.[15] gives an excellent review of some of these methods and modifications, with their advantages and disadvantages.

It may be well to enunciate the requirements of a good determination for blood glucose. First, the test should be simple and take as little time to perform as possible since the glucose determination is probably the most often requested chemical determination. Second, it must show the true concentration of glucose in the blood sample. Both Mosenthal[14] and Haunz[6] have shown that the value of the moiety of non-fermentable reducing substances even in normal bloods is far from a constant and

as much as 78 mg. of the total blood glucose value by the Folin and Wu technic may be non-fermentable reducing substances. Third, the method must be reproducible to enable accurate evaluation of a patient's condition from day to day.

The experimental studies on reproducibility and precision show that the results obtained by the AutoAnalyzer are superior to those found by either the Somogyi or the Folin and Wu deproteinization methods as performed in this laboratory. The better reproducibility of the Somogyi deproteinization method was probably due to the better, more relaxed conditions under which it was performed.

The increased reproducibility of the AutoAnalyzer is due principally to the fact that the sample and standards are all treated in exactly the same way. One measuring device is used to determine the volume of sample, diluent and reagents. This device is the proportioning pump and manifold which remains the same for all samples. Errors due to the difference in calibration of a multitude of pipettes used in the usual manual methods are eliminated and consequently one very frequent source of considerable error is removed completely.

The second criterion is accomplished by the use of physiologic saline as the diluent for the whole blood in the AutoAnalyzer. Since the glucose from the blood sample is dialyzed across the cellophane membrane in the dialyzing chamber of the apparatus the interfering non-glucose reducing substances contained in the red cell are not released to act upon the ferricyanide reagent. Therefore, the glucose measurment by the AutoAnalyzer should give a result very close to that found by the Harding modification of the Schaffer-Hartmann method for true glucose (King, 1951).[10] The normal values for fasting individuals by this method are given as 68 mg. to 96 mg. per 100 ml.[11] The range found by the AutoAnalyzer is from 61 mg. to 95 mg. per 100 ml.

Much time and effort on the part of the technologist is saved by virtue of the fact that once the AutoAnalyzer has been adjusted to a steady base line, the attention required amounts to the filling of the sample plate with the specimens of blood and standards as required. This leaves time for other tests to be started. The whole run may be completed before the graph is plotted and the per cent transmittance of the samples recorded and read from the calibration curve. If the standard curve is run before the samples, the graph may be prepared at once and the glucose value of the samples read as is convenient. In order to check the reproducibility of the curve, it is recommended that frequent standards be interspersed with the unknown blood samples. An occasional sample cup containing physiologic saline is useful in checking the position of the base line throughout the run.

In this laboratory the original manifold suggested by The Technicon Company permitted glucose determinations only as high as 250 mg. per 100 ml. To determine glucose concentrations greater than 250 mg., the 0.5 per cent potassium cyanide reagent used to sensitize the reaction is replaced by 0.9% physiologic saline. A new calibration curve is prepared using a series of standards of glucose concentrations from 100 mg. per 100 ml. to 900 mg. per 100 ml.

Considerable numbers of glucose determinations performed in our

laboratory are from diabetic patients having blood glucose values in excess of 250 mg. per 100 ml. It was found that 15 per cent of the determinations made using the original manifold required repeat determinations using the method described for high glucose concentrations.

The alternative manifold described under Methods, decreases the amount of blood used and increases the amount of ferricyanide reagent available for reduction. This increases the sensitivity of the method from 250 mg. per 100 ml. to 350 mg. per 100 ml. The adoption of this manifold has reduced to 3 per cent the number of determinations requiring the use of the high glucose technic.

For values over 350 mg. per 100 ml., the manufacturer's technic is used in this laboratory in preference to diluting the blood. Diluting would cause increased error due to pipetting and defeats the reproducibility of the AutoAnalyzer.

CONCLUSIONS

The use of the AutoAnalyzer for blood glucose and urea nitrogen determinations has enabled accurate reproducible results of both tests to be available quickly. The two determinations comprise a large portion of the routine analyses made in this laboratory and on occasion two technologists have been fully occupied as much as four hours to complete them. The AutoAnalyzer has been used to do the same number of determinations and the results were reported in two hours, the two technologists being released to work on more complex determinations during this period.

SUMMARY

1. The AutoAnalyzer is described.

2. A method for determining true blood glucose levels using the AutoAnalyzer is presented.

3. Comparisons in reproducibility and precision of recovery were made between the routine laboratory method, a Somogyi filtrate method and the AutoAnalyzer method for blood glucose determinations.

4. The normal blood glucose range of 48 fasting blood samples was found to be from 61 mg. to 95 mg. per 100 ml. by the AutoAnalyzer method.

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PREVENTION AND CONTROL OF STAPHYLOCOCCUS INFECTIONS IN HOSPITALS[1]
(Bulletin 1)*

Knowledge of Current Situation

1. It is clear that throughout the world, in spite of the enormous success of antibiotics (and, as will be mentioned below, to some extent because of this success) there remains an important problem of infections, a problem of special significance for hospitals. This report is concerned with such infections, particularly those caused by the coagulase-producing strains of staphylococcus aureus hemolyticus. The most obvious examples are impetigo and more severe infections in children, puerperal mastitis in recently delivered women, burn and postoperative wound infections, and pneumonia in debilitated patients. The staphylococcus may also be responsible for osteomyelitis, meningitis, septicemias, empyemas, boils and abscesses, otitis media, paronychiae, etc.

   Disease-producing staphylococci frequently implant in the nasopharynx without overt disease, thus producing carriers. Indeed, the staphylococcus carrier-rate is a good index of the level of contamination of the environment.

2. Many hospitals have a serious problem with staphylococcus infections, and all hospitals have a potential problem. Information is inadequate as to the incidence of staphylococcus infections which are acquired in hospitals, but there is evidence that the number of such infections is increasing.

3. There appear to be innumerable strains of staphylococcus capable of producing infections. Many of these staphylococci are susceptible to antibiotics. Some are not. Infections with antibiotic-resistant staphylococci constitute the main difficulty. Hospitals are clearly the reservoir of most antibiotic-resistant strains. Strains from the community at large are predominantly sensitive to antibiotics. The strains carried by patients on admission are less frequently resistant than strains which are acquired in hospitals. Patients who acquire these infections in the hospital are potential spreaders of resistant strains to the community after discharge. One of the major factors in the current situation is the widespread use of antibiotics which eliminates susceptible strains of staphylococcus and leaves uncontrolled the resistant strains[2].

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[1] The Committee on Infections Within Hospitals is charged with the responsibility of recommending American Hospital Association policies and programs on the prevention and control of all infections within hospitals. Because of the general interest in the problem, this bulletin is devoted to the staphylococcus. Two earlier bulletins on Asian Influenza have been distributed. This bulletin was prepared by the Council on Professional Practice's Committee on Infections Within Hospitals consisting of: Dean A. Clark, M.D., chairman; William A. Altemeier, M.D.; C. P. Cardwell Jr.; James P. Dixon Jr., M.D.; Maxwell Finland, M.D.; Horace L. Hodes, M.D.; Martha Johnson, R.N.; and Alexander D. Langmuir, M.D., in consultation with Kenneth B. Babcock, M.D., of the Joint Commission on Accreditation of Hospitals; William H. Stewart, M.D., of the Public Health Service; and others.

*From American Hospital Association, Chicago, Illinois.

4. Certain factors frequently found in hospitalization appear to make patients more likely to acquire such staphylococcus infections: a) routine indiscriminate use of antibiotics, especially for "prophylaxis"; b) long hospital stay; c) contact, direct and indirect, with infected hospital patients, staff members or personnel; d) crowding and inadequacy of facilities; e) prolonged operative procedures; f) prolonged use of continuous parenteral therapy through venipunctures or indwelling plastic tubing.
5. Certain factors in the host (patient) appear to increase susceptibility to infection: a) treatment with adrenocortical steroids; b) physical debility; c) chronic disease; d) prematurity; e) diabetes; f) bed sores; g) open wounds or breaks in the skin; h) chronic pulmonary disease.
6. Danger of infection seems to be especially great from direct exposure to people infected with the staphylococcus, although exposure to the same organisms in or on contaminated equipment, supplies, dressings, air, dust, wall or floor surfaces, linens, etc., may be equally as important. The physician, nurse or other attendant with a boil, paronychia, abscess, or nasopharyngeal infection with a virulent strain is particularly hazardous.

Recommendations

I. All hospitals should establish Committees on Infections, to devote particular attention to infections which are acquired in hospitals so they may be reduced to the lowest possible minimum[3].
   A. It is suggested that the committee on Infections include, where possible, a bacteriologist, a pediatrician, a surgeon, an internist, a nurse, and a hospital administrator. The local health officer should be urged to serve as a consultant to the committee. The committee should report periodically to the executive committee of the medical staff.
   B. The functions of the Committee on Infections should include at least the following:
   1. To establish a system of reporting infections among patients and personnel, such a system being essential to a proper understanding of infections which are acquired in hospitals. The committee should have access to all reports of infections anywhere in the hospital.
   2. To keep records of infections as a basis for the study of their sources and for recommendations regarding remedial measures.
   3. To distinguish to the best of its ability between infections acquired in the hospital and those acquired outside.
   4. To review the hospital's bacteriological services to make sure that such services are of high quality and are accessible either in the hospital itself or in an outside laboratory. Bacteriophage typing, if not available in the hospital, may be sought, as needed, through official local and state health agencies.
   5. To review aseptic techniques employed in operating rooms, delivery rooms, nurseries, and in the treatment of all patients with infections and, if indicated, to recommend methods to improve these techniques and their enforcement.
   6. To make vigorous efforts to reduce to the minimum consistent with adequate patient care.

a) Use of antibiotics, especially as "prophylaxis" in clean, elective surgery
b) Treatment with adrenocortical steroids

7. To undertake an educational program to convince medical staff and hospital employees of the importance of reporting to responsible authorities when they have skin infections, boils, acute upper respiratory infections, and the like.

8. To establish techniques for discovering infections which do not become manifest until after discharge from the hospital, it being known that such infections are often overlooked because they may not be apparent until several weeks after the patient has left the hospital. Two approaches to discovering such infections are suggested:

   a) An attempt to trace the source of any infection with which a patient may be admitted. For example, if an infant is admitted with staphylococcal pneumonia or a recently delivered mother with mastitis, the hospital where delivery occurred should be determined and informed of the infection so that it can seek possible sources of infection.

   b) Periodic telephone polls on a random sample of discharged patients (particularly recently delivered mothers, newborns, and post-operative patients) to ascertain their state of health and, in case of any indication of infection, to follow them up. Such surveys have proved quite simple and quite valuable. A detailed account of the method is given by Ravenholt and others in the October 1956 issue of the American Journal of Public Health.

II. Hospital administration should undertake the following measures to assist in the control of infections:

   A. Diligent maintenance of the general cleanliness of *all* areas in the hospital, not simply in those associated with operating rooms, delivery rooms, and nurseries. Other possible sources, such as dust, air pollution (special attention should be given to ventilating and air-conditioning systems and their filters), and floors must also be considered as potentially important factors in the spread of infection. There should be regular inspections of the hospital for general cleanliness.

   B. Special studies among staff and personnel to uncover silent carriers of staphylococcus, especially in epidemic situations accompanied by repeated cases traceable to the same organism.

   C. Appropriate measures for the treatment of all carriers who persistently show heavy growth of epidemic strains of staphylococcus in nasopharyngeal cultures or who are identified by epidemiological evidence.

[2] It is not known whether resistant strains of this organism actually acquire resistance after exposure to the antibiotics, or are resistant to begin with and are simply unmasked by the suppression of susceptible strains—although the latter is thought to be the case. From a practical point of view it does not matter which theory is correct.

[3] The Joint Commission on Accreditation of Hospitals is being asked to consider the establishment of a Committee on Infections as a major factor in the accreditation of a hospital. Bulletin 17 of the Joint Commission contains recommendations on the subject.

D. Transfer of such carriers and personnel with skin infections, boils, acute upper respiratory infections, and the like from locations such as operating rooms, delivery rooms, food-handling positions, and nurseries to other duty stations in the hospital. Usually such transfers have proved to be sufficient to control the problem, but occasionally leave of absence for a persistent carrier has been necessary.

III. Hospitals should initiate or participate in community programs to control infection through cooperation with other hospitals, local medical societies, local health departments, and other groups.

General Comment

1. Occasionally, an entire hospital, a whole community, or a large area seems to become subject to an epidemic strain of staphylococcus. Why this occurs is not known. Its occurrence, however, points up the need for more general recognition and study of staphylococcus infections.
2. Among the agencies from which consultation and assistance concerning infection problems may be sought are the following:
   a) The American Hospital Association, the American College of Surgeons, and the American Academy of Pediatrics (especially for newborn infants) which will furnish upon request the names of suitable consultants.
   b) Local and state health departments which, in many instances, have experts on their staffs.
   c) The Communicable Disease Center of the U. S. Public Health Service, Atlanta, Georgia, whose assistance may be obtained through local and state health departments.
3. Valuable background information and discussion about the infection problem can be found in:
   a) Conference on staphylococcal infections. (Symposium) Journal of the American Medical Association. 166: 1177-1203, March 8, 1958; (Editorial p. 1205)
   b) Observations relative to the nature and control of epidemic staphylococcal disease. F. H. Wentworth and others. American Journal of Public Health. 48: 287-98, March 1958
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A DEFIBRINATION-ROTATION METHOD FOR DEMONSTRATING LE CELLS*

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A number of techniques for the production of LE cells have been developed, since the LE cell was first described by Hargraves[1] from heparinized bone marrow. These techniques include examination of concentrated leukocytes (buffy coat) of anticoagulated blood, bone marrow mixtures of LE plasma or serum with non-lupus blood or bone marrow, and concentrated leukocytes from clotted blood.[2,3,4,7] The two hour clot test has been shown to have greater sensitivity than the buffy coat from blood or bone marrow preparations.[4,7] Zinkham and Conley[3] found that a method using heparinized blood rotated with glass beads compared favorably with the two hour clot test.

The typical LE cell is a leukocyte, usually a neutrophil, but occasionally a monocyte or eosinophil, whose nucleus is crowded to one side by a large homogeneous basophilic inclusion body. The periphery of this inclusion body does not appear to be sharply defined, as by a membrane, and although the material has been shown to be nuclear in origin by cytochemical means, no nuclear structure is evident. This homogeneous material can often be seen as extracellular bodies on LE preparations, and neutrophilic clumping is often present. The presence of leukocytic clumping and/or unphagocytized homogeneous LE-like material on a preparation is not considered to be definitive diagnostic evidence. It is necessary to distinguish between typical LE cells and "tart" cells which similarly contain inclusions, but whose inclusions are not homogeneous and have retained evidence of nuclear structure. "Tart" cells have no known specific diagnostic significance.

The majority of positive LE cell tests have been seen in patients with acute disseminated lupus erythematosus. Occasional false positive tests have been reported, most frequently in cases of rheumatoid arthritis and drug hypersensitivity.[5]

The presence of anticoagulants tends to inhibit the formation of LE cells and thus to reduce the sensitivity of the test.[6] A minimum amount of heparin, 0.75 mg/10 ml of blood[6] appears to be the anticoagulant of choice if one is used.

Since both coagulation and rotation with glass beads appear to contribute to the sensitivity of LE cell tests, and since anticoagulants have shown to be inhibitory, the combination of the two factors should produce optimal conditions for the formation of LE cells. To devise such a method, blood was defibrinated and rotated with glass beads, and the material obtained was compared with preparations from the two hour clot test.

Materials and Methods

Ten ml venous blood from each subject was put into a 50 ml flask with two to three dozen glass beads, 3 mm diameter. The flask was swirled for 15 minutes to defibrinate the blood and was allowed to stand for another 15 minutes. Five ml defibrinated blood was then transferred

* Claudia M. Stannard Award 1958. Read before the 26th Annual Convention of ASMT, Milwaukee, Wisconsin, June, 1958.

to each of two test tubes containing six glass beads, 3 mm diameter. One-tenth ml thromboplastin was added to one tube. The tubes were stoppered and put on a rotator* (used for mixing hematology specimens) at 26 RPM. The tubes were rotated for 30 minutes, then allowed to stand undisturbed at room temperature for one hour. Both tubes were centrifuged for 10 minutes at 3000 RPM, the buffy coat from each tube removed to a Wintrobe hematocrit tube for greater concentration and recentrifuged for 10 minutes at 3000 RPM. The concentrated buffy coat, with approximately an equal amount of serum, was removed with a capillary pipette, mixed and spread on glass slides. The films were rapidly dried and stained with Wright's stain.

The two hour clot test was used as described by Zimmer and Hargraves.[7] The clot was pushed through a 40 mesh wire sieve**. The sieved material was collected, centrifuged in a Wintrobe hematocrit tube, and the concentrated white blood cell layer spread on glass slides and stained.

Results

Blood from ten subjects was studied with each method. One thousand leukocytes from five slides were counted for each method in order to evaluate the relative numbers of LE cells in each preparation. The counts were done in an area where the white cell concentration was greatest, usually near the feather edge. The results are illustrated in Table I.

Number of LE Cells Per 1000 Leukocytes

Case	Diagnosis	2 Hour Clot Method	Defibrination—Rotation Method	
			Thromboplastin Added	No Thromboplastin Added
1	Acute disseminated lupus erythematosus	129	164	145
2	Acute disseminated lupus erythematosus	56	281	100
3	Acute disseminated lupus erythematosus	14	194	206
4	Acute disseminated lupus erythematosus	0	1	5
5	Rheumatoid arthritis	12	81	112
6	Rheumatoid arthritis (Autopsy diagnosis: periarteritis nodosa)	120	70	197
7	Probable periarteritis nodosa	0	0	0
8	Subacute lupus erythematosus	0	0	0
9	Renal disorder (undiagnosed)	8	27	16
10	Probable collagen disease	0	0	0
11	Normal	0	0	0
	Total	339	818	781

LE cells were present in all preparations (from the two hour clot test and the defibrinated, rotated preparations with and without thromboplastin) from three cases diagnosed as acute disseminated lupus erythematosus, in two cases diagnosed as rheumatoid arthritis, (one of which revealed periarteritis nodosa at autospy), and in one case with undiagnosed renal disease. In one case, diagnosed lupus erythematosus disseminatus, the two hour clot test was suspicious but without typical LE

* Baltimore Biological Laboratory, Inc., Baltimore.
** Scientific Products Division, Am. Hosp. Supply Corp., Chicago.

cells, while the defibrinated preparations with and without thromboplastin were positive with typical LE cells. No LE cells were found in one case diagnosed as periarteritis nodosa, in one case diagnosed as subacute lupus erythematosus, in one case classified nonspecifically as collagen disease, and in one normal subject.

In all cases in which LE cells were found, more LE cells were counted on preparations from the rotation technique than the two hour clot test. No significant difference was noted between defibrinated preparations with and without thromboplastin. In one instance in an undiagnosed subject, small numbers of LE cells were found with the defibrination-rotation method, but none were seen in the two hour clot test.

Discussion

Zinkham and Conley presented evidence that the two hour clot test and the rotation method compare well with regard to sensitivity. Dubois and Freeman found a slightly greater incidence of LE cells in a method modified from Zinkham and Conley's than in the two hour clot test. The results of our study comparing the defibrination-rotation-glass bead technique with the two hour clot test revealed a greater number of typical LE cells formed by the defibrination-rotation-glass bead technique. In one case, small numbers of LE cells were found with defibrination-rotation technique, while typical LE cells were not seen in the two hour clot test. However, since the series is small, no definite conclusions can be made regarding the absolute incidence of LE cells in the two methods.

The LE cells from defibrinated-rotated blood often showed better definition as typical LE cells than similar material from the two hour clot test. The two hour clot test contained considerable amounts of extracellular basophilic material similar to that seen in LE cells, while the defibrination-rotation preparations revealed less extracellular material and more typical LE cells. This seemed to be borne out by the greater number of typical LE cells in the latter method. No advantage appeared to be gained by the addition of thromboplastin.

Summary

1. A modified Zinkham-Conley technique using defibrinated blood rather than heparinized blood was compared with the two hour clot test in ten subjects.
2. In positive preparations more LE cells were produced with the defibrination-rotation technique than with the two hour clot method.
3. In one case, typical LE cells were found with the defibrination-rotation technique but not with the two hour clot test.
4. The defibrination-rotation technique was often more easily interpreted than the two hour clot test.
5. The addition of thromboplastin did not appear to increase the sensitivity for the LE cell phenomenon.

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(Continued on Page XXXVI)

THE QUANTITATION OF SUGAR IN URINE**

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INTRODUCTION

The measurement of the amount of sugar in urine of diabetic patients is an important clinical procedure which is useful in regulating insulin dosage. The amount of sugar in the urine of a diabetic also has some utility in defining the severity of metabolic disturbance.

Copper reduction tests have long been used for quantitation of sugar in urine. The Benedict's quantitative procedure[1] has been used for this purpose but it is cumbersome, requires special equipment, laboratory facilities and technical skill. Over the past 15 years Clinitest*, which is a self-heating copper reduction type of test, has been established as the method of choice for quantitation of sugar in urine. As this test gives quick and accurate results, it has become widely used by physicians, laboratory workers, technologists, nurses and patients. More recently simple strip tests for glucose, based on the use of an enzyme glucose oxidase have been made available. Although the enzyme tests have the advantage of specificity for glucose, there are ample data[2,3] to indicate that they do not reliably quantitate the amount of glucose in urine.

This paper describes a new "Two-Drop" procedure which expands the range over which Clinitest can measure the concentration of sugar in urine. Comparative studies between the new procedure and existing methods are also presented.

METHODS

Several methods for the estimation of urine sugar have been utilized in obtaining data included in this paper. These methods are described below.

Clinitest. The procedure used for Clinitest is that described on the direction sheet which is a part of the package. Five drops of urine and ten drops of water are placed in a small tube and a tablet of Clinitest is added. Fifteen seconds after boiling ceases the tube is shaken and comparison is made with the color chart. With urines containing over 2% sugar the reaction of Clinitest exhibits a phenomenon known as the "pass through." In such cases the boiling fluid develops a series of colors including an orange shade but passes through this color to a dark shade of brown. In these instances the result is recorded as "over 2%" without comparing with the color scale.

Giordano Modification of Clinitest. Giordano, Pope, and Hagan[4] have described an alternative procedure for Clinitest which is used when elevated values are obtained. If an initial value of over 1% and less than 2% is obtained a dilution of one volume of the urine is made with two volumes of a normal sugar-free urine. This diluted sample is retested with Clinitest by using 5 drops along with 10 drops of water. The result of this test is then multiplied by 3 to obtain the sugar concentration. If an initial value of over 2% is obtained, a dilution of one volume of the urine is made with nine volumes of a normal sugar-free

*Clinitest is a registered trademark of the Ames Company Inc., Elkhart, Ind.

**Read before the 26th Annual ASMT Convention, Milwaukee, Wisconsin, June 1958.

urine. Five drops of the diluted urine along with 10 drops of water are used and the result is multiplied by 10 to obtain the sugar concentration.

Schmidt Modification of Clinitest. Vagn Schmidt[5] has recently suggested a method which can be employed with urines containing large quantities of sugar. This procedure involves repeating the test using one drop of urine and 14 drops of water. In this instance the reading obtained is multiplied by 5.

"Two-Drop" Modification of Clinitest. The "Two-Drop" method which was developed as a part of this study is carried out as follows: Two drops (0.1 ml.) of urine and ten drops (0.5 ml.) of water are placed in a small tube and a regular reagent tablet for Clinitest is added. Fifteen seconds after boiling ceases the tube is shaken gently and compared with the special "Two-Drop" color chart which has seven colors ranging in values from 0% to 5%.

Benedict's Quantitative Method. This procedure was carried out following the original description given by Benedict[1] as used in our laboratory.[6] From time to time samples of urine containing known quantities of glucose are titrated to standardize the procedure.

RESULTS

Evolution of "Two-Drop" Technic for Clinitest. With the present Clinitest, sugar values up to 2% can be read directly from the color chart while higher concentrations pass through the orange color and on preliminary testing can be recorded only as more than 2%. There would be significant advantage to a modification of Clinitest which would extend to 5% the range of values covered by the initial test. The "Two-Drop" modification of Clinitest has grown out of an effort to extend the range of sugar concentrations that can be measured and at the same time to avoid the "pass through" phenomenon. By experimental approach it was established that a quite satisfactory reaction and range of colors are obtained when two drops of urine are employed with ten drops of water. In general the range of colors obtained with varying concentrations of sugar are comparable to those seen in the regular procedure for Clinitest. However, it was apparent that for optimal accuracy a new color chart should be prepared. This was done by having approximately 12 operators make comparisons with several possible colors using a large number of urines containing known amounts of glucose. This new chart contains seven colors which are designated as follows: negative, trace, ½%, 1%, 2%, 3% and 5%. The trace color does not carry a quantitative designation but it does provide a convenient label for urines giving reactions which are definitely less than ½% but certainly not negative. Some urines containing ¼% sugar will give the trace reaction whereas others containing ¼% sugar will give a color which more closely matches the negative color.

Consistency of "Pass Through." Both Giordano's and Vagn Schmidt's modifications of Clinitest involve repeat tests on those urines which are recognized to contain high amounts of sugar. Since the accuracy of either modification is dependent upon preliminary recognition of the "pass through" phenomenon, a study was carried out to determine the consistency of its occurrence. Twenty operators ran a total of 400 tests involving 40 different random urine samples with known amounts of glucose added. Each operator was given a series of 20 samples to be

tested as unknowns using Clinitest. Particular attention was focused on whether a "pass through" phenomenon occurred. A summary of these results is presented in Table I. It is quite evident from these data that the "pass through" of Clinitest occurs with complete consistency with urines containing over 2% and does not occur with urines containing less than 2% sugar.

TABLE I

"Pass Through" Phenomenon with Clinitest

		Number of Tests	Number of "Pass Throughs"
Range of Sugar Concentration	0—2%	200	0
Range of Sugar Concentration	3%—5%	200	200

Although the "pass through" did occur with 100% frequency in urines containing over 2%, it was quite evident from this study that in many instances the presence of the orange color was quite fleeting. Accordingly it is easy to understand how the "pass through" of Clinitest might be missed where the tube is not carefully watched during the whole reaction. If one does not see the "pass through" it is quite difficult to recognize its occurrence afterwards since the final color may fairly closely match the color of ¾% or 1% sugar on the color chart for Clinitest.

Comparison of Results with "Two-Drop" and Regular Procedures for Clinitest. Results obtained with the "Two-Drop" modification have been compared with results obtained with the regular procedure for Clinitest. These studies have been carried out on normal urines, on sugar-free hospital urines to which glucose was also added, and on hospital urines already containing glucose. Results are described separately under each heading.

Normal Urines. In order to assess the performance of the "Two-Drop" procedure for Clinitest in testing urines from healthy subjects, it was used in comparison with the regular procedure for Clinitest in a Diabetes Detection Program. A total of 742 samples of urines was tested with each method. The enzyme stick test was used to differentiate between true glucose and nonglucose reducing substances. Results of this study are summarized in Table II. It will be seen that all urines which were negative by regular Clinitest were also negative with the "Two-Drop" technic. Five urines which contained ¼% or less of glucose were re-

TABLE II

Comparison of Two Procedures for Clinitest in Diabetes Detection

Number of Samples	Urine Constituent	Regular Clinitest	"Two-Drop" Clinitest
723	.....	Negative	Negative
4	Nonglucose	½% or less	Negative
1	Nonglucose	½%	½%
5	Glucose	¼% or less	Negative
1	Glucose	¼%	Trace
6	Glucose	½%—2%	½%—2%
2	Glucose	more than 2%	5%

corded as negative with the "Two-Drop" test. In 4 out of 5 instances where nonglucose reducing substances were present the "Two-Drop" test gave a correct negative result.

Sugar-Free Hospital Urines. The object of this study was to determine whether drug metabolites or other abnormal urinary constituents had any adverse effect on the "Two-Drop" modification. A total of 200 hospital urines which were negative with Clinitest was used in this study. All urines that were negative by the regular procedure were also negative by the "Two-Drop" modification. To each of these urines was added ½% and 4% glucose. These glucose urines were then tested by both procedures. Results are given in Table III. When ½% was added, values of ½% were obtained most of the time by both methods. When 4% was added, values of more than 2% were always obtained with the regular procedure and values of 3% or 5% glucose were obtained 88% of the time with the "Two-Drop" modification. Some urines, primarily those with large quantities of protein, gave colors interpreted as 2% with the "Two-Drop" modification.

Hospital Urines with Naturally-Occurring Glucose. A series of 25 urines containing excreted sugar was tested using the "Two-Drop" modification and the regular procedure for Clinitest. The amount of sugar in each specimen was established by using Benedict's quantitative method. Results of this study are shown in Table IV. It can be seen that results with the two procedures for Clinitest show good agreement for values of 2% or less. With higher levels of glucose, the "Two-Drop" modification provides a much closer estimate than does the "more than 2%" result of the regular procedure.

TABLE III

Tests on Sugar-Free Hospital Urines with Glucose Added ("Two-Drop" and Regular Procedures for Clinitest)

Value Reported	GLUCOSE ADDED		
	0 Percent	½ Percent	4 Percent
Regular Clinitest			
Negative	100%		
¼%		6%	
½%		82%	
¾%		12%	
More than 2%			100%
"Two-Drop" Clinitest			
Negative	100%		
Trace		6%	
¼%		93%	
½%		1%	
2%			12%
3%			65%
5%			23%

Results with Giordano's Modification of Clinitest. Various amounts of glucose were added to a series of 100 urines from healthy subjects. These were then tested by an experienced operator. Only urines which tested more than 1% with regular Clinitest were in a range to be retested by Giordano's modification. Table V, therefore, contains data only on high glucose urines. As can be seen from this table, Giordano's

TABLE IV

Tests on Hospital Urines Containing Voided Glucose ("Two-Drop" and Regular Procedures for Clinitest)

Glucose Concentration (Benedict Quant.)	Regular Clinitest		"Two-Drop" Modfication	
	Glucose Reported	No. of Urines	Glucose Reported	No. of Urines
Less than 1 percent	½%	6	trace	1
			½%	5
1—1.9 Percent	¾%	3	¾%	3
	1%	2	1%	2
2—2.9 percent	2%	3	2%	3
3—3.9 percent	> 2%	2	3%	2
4—4.9 percent	> 2%	1	3%	1
5—5.9 percent	> 2%	3	5%	2
			> 5%	1
6—8.4 percent	> 2%	5	> 5%	5
		25		25

modification provides a satisfactory means for the measurement of high glucose concentrations in urine. To derive the advantages inherent in this modification it is essential first to detect the "pass through" during the preliminary testing by the regular procedure for Clinitest. If this "pass through" is recognized and subsequent dilution is correctly made and calculated, then accurate results can be obtained.

TABLE V

Tests on Normal Urines with Glucose Added (Giordano's Modification of Clinitest)

Percent Glucose Added	Frequency of Reported Glucose Values			
	1.5 Percent Glucose	2.25 Percent Glucose	2.5 Percent Glucose	5.0 Percent Glucose
2 Percent	72%	28%		
3 Percent			71%	29%
4 Percent			63%	37%
5 Percent			18%	82%

Results with Vagn Schmidt's Modification of Clinitest. In a small series of studies a trained operator ran tests using Vagn Schmidt's technic. The method primarily gave values which were comparable to the results obtained with the Giordano dilution procedure. However the quality of the color was not always completely satisfactory. With this technic a preliminary test is first run by the regular procedure for Clinitest so again the usefulness of the test depends upon recognition of the occurrence of the "pass through" with high sugar urines.

Quantitation of Sugar by Inexperienced Operators. Twenty-five inexperienced operators ran urine sugar tests using several different methods. A total of 150 tests were run by each method. When using Benedict's quantitative method these inexperienced operators obtained unsatisfactory results and frequently made gross errors. Yet Benedict's quantitative test in the hands of experienced operators will give very accurate results. When using Clinitest or modifications of Clinitest, these inexperienced operators obtained entirely satisfactory results with urines

containing 2% glucose or less. With very high sugar urines gross errors occurred due to missed "pass through," errors in making dilutions, and errors in calculating results after dilutions. When using the "Two-Drop" modification of Clinitest these inexperienced operators rarely made errors and obtained satisfactory results with all levels of glucose concentrations. Their results were comparable to those obtained by experienced operators.

DISCUSSION

The amount of sugar excreted in the urine in diabetic patients who are inadequately regulated is influenced by many factors. The majority of sugar-containing urines have concentrations ranging from ¼% to 2%. However, a certain number of urines from diabetic patients will have glucose concentration between 2% and 5% and on rare occasions, concentrations between 5% and 10% will be encountered. The modified procedure for Clinitest was evolved on the basis that it would give an optimal range of coverage of sugar concentrations with a maximal accuracy. The data obtained in the diabetes detection study show that the sensitivity of the "Two-Drop" procedure is not significantly different from the sensitivity of the regular procedure for Clinitest. This is due to a decrease in the amount of interfering substances with the use of less urine. The modified procedure extends the upper range to include most concentrations which will be encountered in urines containing "large amounts" of sugar. The infrequent urine with 7% or 8% sugar will exhibit the "pass through" phenomenon with the "Two-Drop" procedure. However, this can be readily seen and even if the "pass through" is not noted the final concentration will be read as 3% which is regarded as a large amount of sugar by all present methods.

The amounts of urine and water employed in the "Two-Drop" procedure were selected as quantities which give good reactivity with tablets of Clinitest and as quantities which can readily be measured either with a pipette or with a dropper. In comparative studies with the "Two-Drop" procedure no significant difference in accuracy was revealed when droppers or pipettes were used to make either or both liquid measurements. This may be interpreted to indicate that with "standardized" droppers the accuracy of drop measurement is not a limiting factor in the overall accuracy of the method. However, if droppers are used which do not deliver 20 drops per milliliter or if droppers are used carelessly then it is desirable to make these measurements with a pipette. In many laboratories pipette measurements tend to receive more attention than drop measurements. In fact, in a laboratory where several tests are being made some sort of automatic dispenser for ½ milliliter quantities of water is quite useful.

It has long been recognized that urine contains a substance which interferes with the copper reduction reaction given by glucose. Accordingly, it is not surprising that with a reduction in the amount of urine employed with Clinitest, the resulting colors are somewhat different. The decrease in the total reacting volume also causes some change in the colors. The color chart (7 colors) for the "Two-Drop" modification of Clinitest cannot be used with the regular procedure nor can the color chart (6 colors) for the regular procedure be used with the "Two-Drop" modification.

Urines containing protein behave in an atypical manner when tested with Clinitest. These urines give more foam and boil very slowly. Protein in the urine causes no alteration in the qualitative accuracy of Clinitest in detecting reducing sugar, but it may have some effect on the quantitation of sugar. This effect is seen in the experiments in which glucose was added to urines from hospital patients since those which contained protein gave somewhat lower values than would be expected with the "Two-Drop" method.

Certain advantages of the presently described "Two-Drop" modification of Clinitest appear in comparison with the modifications by Giordano[4] and by Schmidt.[5] Either the Giordano or Schmidt procedure depends on first running a Clinitest by the regular procedure. As has been indicated above if the urine contains over 2% sugar it will exhibit the "pass through" and in some instances this may not be seen either due to its fleeting nature or due to the attention of the operator being directed to some other aspect of his work. In contrast the "Two-Drop" method does not require this same careful attention during boiling. A second advantage of the "Two-Drop" procedure is that it does not require a rerun with urines containing large amounts of sugar. The calculation involved in reruns with either the Giordano procedure or the Schmidt procedure can introduce a possible error, particularly with inexperienced personnel. In the Giordano procedure the "sugar-free" urine employed for dilution involves an additional reagent and an additional test. This is not a factor in the Schmidt procedure but with this method the employment of only one drop of urine in the rerun may result in a color which does not afford optimal comparison with the color chart.

SUMMARY

A simple "Two-Drop" modification for Clinitest is described. This provides an improved means of quantitating urine sugar over a wide range of concentration from trace to more than 5%. Data are presented to show that the method is accurate and sensitive. It performs satisfactorily with random urines collected from hospital patients. The simplicity of the method insures a minimal number of errors either by experienced or inexperienced personnel. With inexperienced personnel the quantitative accuracy of the "Two-Drop" modification of Clinitest is superior to that of Benedict's quantitative method.

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THE JOURNAL CLUB IN MEDICAL TECHNOLOGY*

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Rapid advances in the biological sciences are continuously presenting medical technologists with new ways to aid surgeons and clinicians in the diagnosis and treatment of human disease as well as with more accurate, economical, and specific methods to achieve this end. In our search for an adequate means of knowing and selecting the procedures which filled our own needs we chose a modification of the "Journal Club" used in the medical and biological sciences of St. Louis University Graduate School.

The Journal Club at the University was a separate function for each department electing to offer this type of seminar. The members consisted of professors and graduate students of the department, students taking minors in the department, and anyone else who wished to attend. At the weekly sessions one of the members presented a report on any article from a current journal, gave a resumé of other material on the same subject, and then contrasted or enlarged it with the work of other authors. At the conclusion of the report there was an open forum with questions, comments, and varied interpretations by all members of the club. This obviously alerted the students to recent developments within the discipline and gave them the advantages of hearing the professors and assistants critically discuss and evaluate the material presented.

Each Journal Club can, of course, determine its own limitations of time, of presentation, discussion, and scope of material. In formulating our own club for the laboratory staff, we eliminated the requirement of obtaining the historical development and obsolete modifications of the technics employed, for although we realized the educational importance of the information so gained, we felt that none of us had the time for prolonged search in older journals nor a practical application for the knowledge of currently non relevant developments. Instead we substituted a comparison with current technics and the clinical application and/or advantages accruing to the patient, the clinician, or the medical technologist.

There are a number of ways of selecting the subject matter for the sessions. The most elastic method for a group with mixed interests might be called the "arbitrary" method. In this each member is assigned a date; she chooses any article of interest to her, posts a notice of the subject, references, and the date of the report at least one week in advance. This preposting enables the other members to familiarize themselves with the material sufficiently to discuss it intelligently. This method is the most popular with our own staff since the group is composed of students, young graduates rotating in the various departments, and the experienced technologists supervising the biochemistry, microbiology, serology, histology, hematology, and blood bank divisions. One week we may concern ourselves with the use of frozen leucocytes for the L-E phenomenon, and the next week participate in a discussion

* Read before the 26th Annual Convention of ASMT, Milwaukee, Wisconsin, June, 1958.

on cryoglobulinemia or a comparison of the various methods for drug sensitivity of microorganisms.

Another method of selecting the material for the Journal Club meetings is for the members to choose some area which is giving difficulty, needs adjustment to newer concepts, or is not fully explored by the group. Each one then works out an assigned portion of the problem, and in turn, presents his facet to the group. At the end of the individual reports the respective portion of the problem is discussed, and at the conclusion of the entire unit, the whole is reviewed, commented on, and the application to our own laboratory decided on. This spring we intend to study methods of controls in laboratory analyses and have allocated our material into a study of graphic control of daily variations, recovery of added substances, the use of standards prepared by the American College of Clinical Pathologists and various commercial sources; blood bank controls; and one of us has consented to explain statistical controls.

A third method of selecting subject matter for the sessions of the Journal Club is to vote for study units with presentations on various phases of the unit. Electrophoresis, we discovered, had applications in hematology, serology, and biochemistry. Sickle Cell Anemia could be explored in its hematological, biochemical, and pathological aspects. This type of study usually elicits the greatest amount of discussion, but needs a readiness of the members to follow the leadership of a chairman in organizing the topic breakdown as well as in monitoring the sectional discussions in order to forestall a premature "full and free" exposition of some individuals "conclusions" before all the units have been presented.

A Journal Club, of course, needs journals. The Administrator of our hospital has been most generous in supplying journals for all the departments, and thus our laboratory staff has an unusual number of them at their disposal. We were somewhat surprised then to note that the majority of reports during the past eight years have been based on articles selected from the following:

American Journal of Clinical Pathology
American Journal of Medical Technology
Blood
Clinical Chemistry
American Journal of Public Health
Journal of the American Medical Association
Journal of Laboratory and Clinical Medicine
Proceedings of the Society of Experimental Biology and Medicine
Public Health Reports

In addition we have selected topics from Abbott's *What's New,* Ciba's *Clinical Symposia, Lederle Bulletin,* and Sharpe and Dohme's *Seminar.*

One of the problems posed in establishing a Journal Club is the simple one of "When." It is not so simple to answer the question and we did not attempt to. We merely made the announcement, set a time and a place. Strangely enough, everyone concerned soon learned that from two to three on Thursday afternoons, September through May, the laboratory is closed. The entire staff has even moved over to a classroom for the occasion. The secretary answers the phone and is instructed to summon a technologist if a real need arises, and we agreed among

ourselves that the member who presented the preceding week's discussion will answer the call.

The objective of the Journal Club is the important one of keeping abreast of the ever advancing boundaries of medical technology. This is advantageous to the technologist, to the laboratory itself, and to the patient.

The technologist benefits educationally and psychologically. She is forced to read her journals instead of saving them for the proverbial "rainy Sunday afternoon." She is subsequently in a position of knowing newer technics; is able to utilize more rapid, specific, or accurate methods; and is alerted to newer trends in medical technology. She achieves a sense of mastery over her work and a great feeling of belonging to the group. She is completely aware of this and derives self-confidence, poise, and a stimulus to perfect her service to the patient and the clinician. Naturally, any technologist could do this for herself, and while a few of us do, it isn't quite customary.

The student technologist profits by the correlation of various disciplines of medical technology involved in an individual case. She draws on her knowledge of all the phases being taught in order to organize her presentation. She also benefits by the various opinions and interpretations of senior technologists; and learns to evaluate methods and tests in terms of her own laboratory, patients, and doctors.

The laboratory as an organization profits greatly by the spirit of good will, understanding, and integration of work. Everyone seems to be cognizant of each other's problems, needs, and abilities. There has been an ever growing tendency to ask each other's opinions and to confer among themselves on the daily problems that arise. The pathologist is in a position to offer the clinicians new tests, better methods, and more complete analyses at the desirable time. Administration may even discover that the technologists are devising ways and means of solving difficulties on their own initiative.

The advantage to the patient and the clinician are obvious. It has been most gratifying to us that the doctors are coming to the laboratory with a "problem" case and asking if we have come across something to help him establish a diagnosis—or could we set up an array of tests that would assist him in controlling the patient during a new treatment he wishes to try.

PSYCHOSES ASSOCIATED WITH THE INFECTIOUS DISEASE SYPHILIS*

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There are a number of brain disorders and mental illnesses, along with other organic conditions affecting the central nervous system, that give rise to behavioral disturbances. A number of these conditions result from bacteria and viruses. It has been estimated by Coleman[6] that there are approximately 13 million people in the United States suffering from some type of disease or injury to the central nervous system. These, he states, constitute about one-half of all first admissions to mental hospitals.

The disorders associated with brain pathology may be classified as either acute or chronic. Among the acute type are the temporary symptoms including delerium, delusion, hallucinations, general mental turmoil, etc. However, among the more serious chronic type are the syphilitic diseases.

In many of the chronic infectious diseases, of which syphilis of the brain (paresis) is of prime interest, the primary damage may appear to be astonishingly slight and may go completely unnoticed by the patient, relatives, and friends for many years until later in life when the deterioration of mental and motor processes becomes quite evident. The nature and severity of these symptoms depends, of course, upon the nature and extent of the brain damage, but usually includes:[6,17]

I. Changes in mental capacity, as shown by impairment of memory, defects in retention of facts, inadequacy of information, loss of orientation, impaired judgment.

II. Emotional lability or instability, with general emotional oversensitivity and arousal to laughter or tears with minor provocation.

III. Changes in behavior, which most often includes deterioration in conduct and behavior standards, with carelessness in personal habits, loss of interest in occupation and family, decline in ethical standards, and the commission and lowering of moral standards.

IV. Sensimotor disturbances, which may include aphasia (loss or impairment of ability to communicate and understand), paralysis, incoordination, and many other neurological manifestations.

General Paresis

General syphilis is a chronic infectious disease which has taken an enormous number of human lives, and unless properly treated eventually disables and kills its victims. Like tuberculosis, it strikes at the most vigorous and productive age, usually between twenty and forty years. It has been said that if every acute case of this disease were reported, it would be more common than scarlet fever, probably twice as common as tuberculosis, and more than one hundred times as common as infantile paralysis.[22]

Coleman[6] has estimated that about 3%, as compared with previous percentages of 5-10%, of all untreated syphilitics become paretic. General paresis constitutes 1.8% of all first admissions to mental hospitals, declining from 7,187 in 1932 to 1,278 in 1955. (See Table I)

* Read before the 26th Annual ASMT Convention, Milwaukee, Wisconsin, June, 1958.

TABLE I[3]

Syphilitic Psychoses, Reported by Diagnosis, in State Mental Institutions, Continental U. S. 1932-1957

YEAR	Total Syphilitic Psychoses	Percent of Total Psychoses Due to Syphilis
1932	7187	11.00
1933	8043	11.00
1934	8813	11.00
1935	9034	10.00
1936	8884	10.00
1937	9146	10.00
1938	9324	10.00
1939	9531	10.00
1940	8431	9.00
1941	8763	9.00
1942	8576	9.00
1943	7997	8.00
1944	7727	7.00
1945	6546	8.64
1946	6076	7.84
1947	5524	7.23
1948	4817	6.24
1949	4280	5.35
1950	3454	4.32
1951	2816	3.56
1952	2357*	2.99
1953	1954	n.a.†
1954	1520	n.a.†
1955	1278	n.a.†
1956	n.a.†	n.a.†
1957	n.a.†	n.a.†

* Excludes Massachusetts, Wisconsin, South Dakota, and Alabama.
† Not available.

Discovery of General Paresis

Syphilis was unknown in the civilized world before 1493. Soon after 1495 it had spread throughout Europe in a great epidemic. Since then it has existed everywhere. There is substantial evidence that it was carried to Europe by the crew of Columbus on the return of his first voyage.[13]

In 1837 the Frenchman Donne saw corkscrew-like organisms in matter taken from the initial sores of syphilitics. He described these organisms "vibrios", as he termed them, and affirmed that he found them only in the syphilitic chancre.[9]

Several others attempted to prove the cause and cure for this dreaded disease but never succeeded. Salvarsan "606" (arsphenamine), the major chemical weapon which Paul Ehrlich, a German-Jew, had forged in his laboratory, was known to bring healing to some syphilitics but never to paretics. Emil Kraeplin sought to prove the equation Syphilis + alchohol = paresis. He, however, failed to do so.[11]

In 1905, the causative organism was discovered by Fritz Schaudinn, a protozoologist, and Eric Hoffman, a dermatologist, at the University of Berlin. This they named "Spirochaeta pallidum" because of its low refractive power, but later changed it to "Treponema pallidum." Thus with this discovery began the search in the brains of paretics for the spirochaete.

In 1913 Hideyo Noguchi, the great bacteriologist who perhaps knew more about spirochaetes than anyone else alive at the time, detected what he thought were spiral-shaped organisms in about 7 of 200 slides

of paretic brain material. This organism was found in the nerve cell layers of the paretic cortex, thus at last accounting for the tissue destruction recognized but not explained nearly a century before. The cause of paresis was now definitely ascertained. It remained to find a remedy.

Development of General Paresis

General paresis, general syphilis, general paralysis of the insane, and *Dementia paralytica* are all terms given to the mental manifestations of syphilis. This is the pitiful end for a number of patients with untreated syphilis.

The causative agent, *Treponema pallidum,* is an extremely tiny (4-14 microns long), delicately weak, actively motile, long spiral or corkscrew structure tapering to a flagellum at both ends and having 4-15 tight corkscrew spirals. It is a very strict parasite and its life outside the animal body is short. It is susceptible to heat and dies rapidly in stored blood. This organism damages and destroys the tissues of the brain and spinal cord by progressive infiltration. When invasion of the organism occurs in the cerebral cortex to a considerable degree, *psychosis* is likely to occur.

Perhaps the main reasons why this organism had escaped detection for so long are that (1) it is not stained by the aniline dyes used to demonstrate other bacteria (2) it grows only "in vitro" under special conditions (3) the organism is present only in large numbers in active lesions, and it has been obtained in pure culture from them, and (4) the disease has been produced in animals from which the organism has again been cultivated. Thus Koch's postulates have been filled by this spirochaete.

Natural History.

From the beginning syphilis is always a generalized blood stream infection. There are many different ways the corkscrew-like spirochaete may gain entrance to the body. Syphilis is typically spread from person to person during sexual intercourse, although it may be spread in a few cases by kissing. Other ways of being infected are by minute breaks or scratches in the skin or by direct contact with open syphilitic sores or lesions. The danger to surgeons and dentists of infecting their fingers through cuts and abrasions while working on syphilitic patients is considerable. All manual examinations on syphilitics should be made with gloves.

There are four stages in which the spirochaete begins its systematic destruction:

First Stage. The spirochaetes enter the blood stream within a few minutes after innoculation and spread throughout the body within 24 hours. Immediately they begin to multiply rapidly, entering first the lymph capillaries, and then are carried to the nearest lymph gland which acts as a temporary filter and incubator. There is then the primary incubation period lasting an average of 10-40 days. At the end of this period there appears at the site of infection in many patients, but not all, a so-called "primary lesion" or *hard* chancre. Since the chancre is the first recognizable lesion it is most important that it be seen and identified. Although it may take various forms, the uncomplicated lesion often appears first as a papule which grows and becomes a smooth, regularly

bordered ulcer that has a punched out appearance. If uncomplicated, it is painless. In some instances it may be so insignificant that the patient and his friends or family is completely unaware of its existence. Even when untreated, the sore disappears in from 4-6 weeks, often leaving the patient with the mistaken notion that it was only a minor irritation or that now he is completely cured. The lesion is usually single rather than multiple and may be accompanied by enlargements of adjacent lymph nodes (satellite buboes).

Second Stage. From approximately 3-8 weeks after the chancre has disappeared a rash appears. This may cover a portion, and in some cases, the entire body. To the inexperienced it may appear to be measles or smallpox. For this reason the term "Great Pox"[13] was used to differentiate the disease from others. This rash is accompanied by mucous patches in the mouth and throat which are almost pure cultures of the spirochaete. Fever, headache, indigestion, loss of appetite, and other symptoms may accompany this stage.

It is during this second stage that a relative balance between the infectious agent and the tissues of the host takes place. Here a large majority of the organisms are eliminated. Those that remain become localized, often to make trouble later as the various tissues become allergic to them. Therefore, it may be stated that the early lesions contain *many* spirochaetes and are dangerous to others but not to the patient, whereas the late lesions contain few spirochaetes and are relatively non-infectious but are extremely dangerous to the patient because of their destructive nature.

Third Stage. This is known as the early latent period, for now all symptoms of the disease disappear. This is probably the most dangerous period, for while the patient discontinues treatment on the idea that he is cured, the spirochaetes are busily attacking the various internal bodily organs, causing permanent degeneration. It is a known fact that *Treponema pallidum* takes advantage of the lowered resistance of each and every tissue it comes in contact with. Blood vessels and nerve cells, especially of the brain, seem to be favorite targets.

Fourth Stage. Here one sees the total accumulated damage produced during the eary latent period. Now the degenerative power of *Treponema pallidum* shows itself in the form of a sudden heart attack, loss of vision (15% of all blindness is due to syphilis),[6] loss of motor coordination, and the mental disturbances previously mentioned. Sir William Osler (see footnote 1) has tagged syphilis "The Great Imitator" because in its early stages it simulates almost every disease known to man. He once stated, "Know syphilis and the whole world of medicine is opened unto you."

Diagnosis.

During the last century syphilis has become understood mainly on the grounds of clinical, pathological, and serological findings.

Paresis had been described as a disease entity in insanity as early as 1798 by Haslam (see footnote 2) who noticed among patients at the

[1] Osler, Sir William, *Shadow on the Land*, New York, Reynal and Hitchcock, 1937.
[2] Haslam, J., *Observations on Insanity*, London, F. and C. Rivington, 1798, p. 259.

Bethlehem Hospital, a frequent association of delusions of **grandeur,** dementia, and progressive paralysis. He characterized these patients as follows:

"Speech is defective, the corners of the mouth are drawn down, the arms and legs are more or less deprived of their voluntary movements, and in the majority of patients memory is materially weakened. These patients as a rule fail to recognize their condition. So weak they can hardly keep on their legs, they still maintain they are extremely strong and capable of the strongest deeds."

Today, the problem of syphilis is chiefly concerned, in the absence of early syphilis, with the evaluation of positive serologic tests for syphilis.

The serological findings in general paresis are extremely important. (See Table II)

TABLE II

General Classification of Spinal Fluids According To The Degree of Abnormality Observed On Examination By Each of The Five Indicated Tests[10]

Group	Cells/cu mm	Colloidal Benzoin or Gold Curve	Wasserman Reaction	Globulin Test	Total Protein Mg/100cc Average
I*	8-30 small lymphs	0003330000	Negative or weakly positive	pos.	50
II*	30-100 large lymphs	0003330000	Positive or strongly positive	pos.	60
III*	30-100 small lymphs; 10-30 large lymphs; 10-30 polys	5555543210	Strongly positive	pos.	100

*Group I includes fluids in which the number of cells and content of globulin and protein are increased, the flocculation reaction may be negative or positive and the colloidal gold curve may be indeterminate or positive. If positive, the curve is usually of the syphilitic **zone** type.

Group II includes fluids in which the cells number 30 or more/cu mm, the flocculation reaction is strongly positive and the colloidal curve is the **tabetic** or indeterminate type. The estimate of globulin is positive and the value for protein is increased to an average of 60 mg/100cc. Fluids falling just short of the requirements of the severe group also are included in this group.

Group III includes those fluids that have the so-called **dementia paralytical** formula, that is, marked excess of globulins; content of protein averaging about 100 mg/100cc, a strongly positive flocculation reaction (0.2 cc or less) and the type of colloidal curves indicating dementia paralytica. The number of cells is decidedly increased. And, in addition to the small lymphocytes, large lymphocytes and polymorphonuclear leukocytes may be present. However, general paresis does not develop in all cases in which this type of spinal fluid is present.

There are many different ways of detecting the organism. The surest way of differentiating the hard chancre from other and benign ulcers is by a laboratory test known as *darkfield illumination.* Here the silvery spirochaetes may be seen wriggling against a dark background under a microscope with an attachment giving an indirect light. The organism may be detected by this means in the serum drawn from the base of the chancre although the blood may not yet show a positive serologic reaction. It is by this test that the technologist greatly aids the physician in diagnosing the disease in the first hours or days afer he chancre appears, in wha is called the seronegative stage. In the absence of finding the spirochaetes by this method, a complete physical examination may disclose suggestive lesions.

The blood serologies include many different tests, among which the Wasserman by name is perhaps the best known. This complement

fixation test, however, has given way to the easier and less expensive flocculation tests which include the Kahn, Kline, Mazzini, Hinton, Eagle, VDRL, etc. At the present time a new test, called the Rapid Plasma Reagin Test, shows great promise. This test uses plasma instead of serum, is more sensitive than the commonly used VDRL, and is just as specific for syphilis.

There are at least five tests that should be performed on the spinal fluid. These include: (1) Cell count (2) Serologic Test (3) Colloidal Gold Test (4) Globulin Test (5) Estimation of Total Protein. (See Table II)

In 25% of cases the serologic reaction of the blood may be negative in the presence of a positive reaction on the spinal fluid. Therefore it is a necessity to examine the spinal fluid of each patient who has early or latent syphilis, irrespective of the findings of the blood.

Autopsies of paretics reveal thickened brain membranes and marked reduction in normal brain weight and size. Microscopic sections reveal destruction of the ganglion cells and changes in the types of cells.

Treatment.

Before the use of penicillin, treatment consisted in the administration of preparations of arsenic and bismuth, sometimes mercury, and sometimes iodides. In 1943 penicillin was discovered to be effective treatment and at the present time seems the choice treatment for syphilis.

It is now known that the earlier the treatment with penicillin the better the results. When treatment is started during the seronegative chancre phase, that is, before the results of serologic tests have become positive, there is likelihood of "cure" in 90%. This treatment is based upon approximately 10 million units of penicillin. A second course, same amount, will adequatey treat 90% of the remainder, bringing the total up to 99%. As the disease develops to the secondary stage the incidence of successful results drops to about 70%. Therefore, efforts should be made to spot the primary lesion as soon as possible so treatment may be started at once.

Fever therapy is another form of treatment that may be used. This is mainly institutional treatment. Ordinarily it involves the innoculation of the patient with malaria, but now various other methods of artificially induced fever have been developed. These include: (1) diathermy—which involves placing the patient in an electrically-heated cabinet; (2) radiothermy and inductothermy, based upon electronics, and (3) vapotherapy, which involves the circulation of air highly saturated with warm water. A combination of penicillin and fever therapy is apparently even more effective than either one singly.*

*Since this paper was written, it has been reported that cortisone, administered by mouth (100 mgms), or intramuscularly for 1-5 days or even longer has been used in the preliminary treatment in various stages of syphilis. It has often been concluded that cortisone prevents the Herxheimer reaction, so often accompanying the administration of antisyphilitic agents. *Treponema pallidum* had often disappeared from the primary and secondary lesions, and the titer of reagin in the serum of primary and secondary syphilis was either reduced or halted in its usual

rapid rise. In certain cases of tertiary syphilis there was also a decrease in the titer, but in most cases of latent syphilis with a low titer there was no fall after the administration of cortisone. It has also been concluded that cortisone does not appear to modify the sensitivity of *Treponema pallidum* to penicillin and it was of no particular value in hypersensitive subjects with a history of penicillin sensitivity. (MEDICAL SCIENCE, April 10, Vol. 3, No. 7, page 403)

Prognosis.

The problem of syphilis today has changed much in character from the problem it presented about a decade ago. For many communities, and for many countries on the whole, syphilis is the number one health problem. During the calendar year 1956 there were 131,525 cases of syphilis diagnosed and treated.

While a tremendous reduction in the incidence of syphilis has taken place, the disease is by no means eradicated and physicians and public health officials must always try to be alert to the fact that syphilis may produce disability, financial ruin, dementia, and eventually death. (See Table III)

TABLE III[5]

SYPHILITIC CASES REPORTED TO THE PUBLIC HEALTH SERVICE

Fiscal Years 1947-1957

(Known Military Cases Excluded)

In Continental United States

FISCAL YEAR	Total Syphilis	Primary and Secondary	Early Latent	Late and Late Latent
1947	372,963	106,539	107,767	121,980
1948	338,141	80,528	97,745	123,972
1949	288,736	54,248	84,331	121,931
1950	229,723	32,148	64,786	112,424
1951	198,640	18,211	52,309	107,133
1952	168,734	11,991	38,365	101,920
1953	156,099	9,551	32,287	100,195
1954	137,876	7,688	24,999	93,601
1955	122,075	6,516	21,553	84,741
1956	126,219	6,757	20,014	89,851
1957*	135,542	6,283	20,346	100,514

* Provisional.

Freeman[8], reports that the incidence of syphilis, at least early syphilis, is most prominent along the transportation routes of any nation. Figure 1 shows the prevalent regions in the U.S. along with the total syphilitic deaths due to general paralysis of the insane.

Freeman also states: "The amount of early syphilis is considerably more than most people believe or expect. The actual numbers reported depend upon the urgency of case finding techniques. However, since the disease is fairly readily treated with penicillin, and since many people receive penicillin for other kinds of illnesses which may also arrest or wipe out a syphilitic infection about which they know nothing, the incidence of central nervous system syphilis is greatly decreasing."

Figure 1

Region	States	Total Paretic Cases
I	Conn., Mass., N.H., R.I., Maine	19
II	Del., N.J., N.Y., Penn.	99
III	Ken., N.C., Va., W. Va.	80
IV	Ala., Fla., Ga., Miss., S.C., Tenn.	45
V	Ill., Ind., Mich., Ohio, Wisconsin	105
VI	Iowa, Kansas, Minn., Mo., Neb., S.D.	45
VII	Ark., La., N.M., Okla., Tex.	44
VIII	Colorado, Utah	4
IX	Arizona, Calif., Wash.	24
	Total	465

* Latest available statistics.

Maintenance of persons with psychoses due to syphilis supported in mental institutions is estimated to cost $1,107 per person per year. (1955 estimation). These paretics have an average stay of 10 years in the mental institution. In 1957 the total funds spent for this venereal disease totaled $19,237,957.

There have been widespread recent advances in the control and prevention of syphilis, such as free diagnosis and treatment, the requirement by most states of a careful physical examination including the serology before marriage, and various public educational campaigns vigorously promoted by federal, state, and local civic, religious, and educational agencies. Although such action is gradually bringing the disease under control, it still remains a major health problem!

Summary

The symptoms and general dynamic patterns in general paresis have been shown to be not essentially different from those of many other psychotic reactions. The cerebral damage caused by *Treponema pallidum* results in general lowering of adaptive controls and eventually in the severe impairment of intellectual functions. Investigators in the field

have suggested different answers to the cause and cure of this disease. Laboratory tests have helped much in solving many of these. However, the final ones to these questions as yet are not available.

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THE MEDICAL TECHNOLOGIST'S ROLE AT AUTOPSY*

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Many and varied duties are assigned to the Medical Technologist in a smaller hospital. Not the least of these is the service she can render to the pathologist at necropsy. In larger institutions where the services of pathology residents, interns or externs are available, the technologist's duties would be limited. In the smaller institutions her assistance may be sine qua non. She not only performs procedures which are helpful in autopsy diagnosis but may be called upon to serve as "diener" to the pathologist. As the pathologist's assistant or "diener" at the necropsy table, we have found that the technologist must be thoroughly acquainted with the instruments used by the pathologist. She must also acquire a certain degree of skill and proficiency in the performance of her duties as assistant so that she may anticipate the pathologist's requests and facilitate a rapid and complete dissection.

When serving as necropsy assistant, her first duty is to see that everything is in readiness before the pathologist arrives. She should first check the body and remove all clothing and bedding from the corpse. The identification tags should be checked and the patient's hospital record and signed autopsy permit should be laid out in a convenient place for scrutiny by the pathologist. The pathologist's needs can be anticipated as the routine autopsy will follow a pattern. We have found the following to be the usual dissecting instruments needed at necropsy:

1 pair straight dissecting scissors
1 pair curved dissecting scissors
1 pair enterotome scissors
1 pair rib shearing forceps
2 pairs tissue forceps with and without teeth
Several pairs of straight and curved hemostatic forceps
2 Bard-Parker type scalpels
Several large knives
1 probe

Head autopsy:

1 mallet type hammer with hooked handle
1 Virchow chisel
1 hand saw or electric autopsy saw

Supplementary equipment needed should include:

1 large specimen bottle containing 10% formalin
2 large sponges
Paper towels
1 large shallow basin
Post-mortem suturing needles
String
1 large cloth or cellophane bag for organs
Dissecting board
Rubber gloves
Plastic aprons

* Read before the 26th Annual ASMT Convention, Milwaukee, Wisconsin, June, 1958.

The instruments and equipment should be laid out in a convenient fashion to suit the individual pathologist. As the autopsy progresses the technologist can anticipate the pathologist's demands and have each instrument ready for use. The area should be kept clean and as free from excessive blood as possible during the procedure. She can conclude her duties by cleaning the body cavities, replacing the bag of organs and suturing the autopsy incision. The body should then be cleaned and dried in preparation for removal by the undertaker.

The technical duties of a technologist are manifold. These fall into the spheres of bacteriology, hematology, serology, toxicology, chemistry and histology. In each instance proper equipment is needed to perform accurate and dependable examinations. Procedures performed on necropsy material are to be regarded no differently from those done on patients. Accuracy should not be sacrificed just because it is autopsy material that is to be examined. A great deal more may be dependent on the results of tests done at autopsy than on material from the living patient. Aseptic technique is used at the bedside and no less sterile technique is to be used in the autopsy room. The equipment necessary for laboratory tests on autopsy material should be kept on a tray in the autopsy room so that it is readily available to the pathologist and technologist. We believe the tray should contain the following:

Alcohol lamp with matches
Sterile syringes and needles
Sterile test tubes and swabs
Sterile Petri dish and glass slides
Blood culture media
Blood specimen bottles with various anticoagulants
Sterile spinal needles
Sterile cotton, 70% alcohol and merthiolate

Numerous laboratory examinations may be performed on autopsy material. A large percentage of the tests done on the hospitalized patient can also be done on autopsy material. The usual tests done however, are limited to the following:

Smear and culture of inflammatory material
Blood and spinal fluid cultures
Chemical examinations of the blood
Bone marrow analysis

The specimens removed for these tests are handled in exactly the same manner as on clinical material from the living patient. Cultures of inflammatory processes may be taken from anywhere in the body such as in cases with peritonitis, pneumonia, meningitis or abscess cavities. Sensitivity tests done on organisms from such cultures may prove the infecting organisms to be resistant to all the usual antibiotics and therefore the cause of death. Blood cultures and cultures of vegetations from the heart valves can document a case of subacute bacterial endocarditis. Cultures of a purulent spinal fluid can verify a suspected meningococcal meningitis. Chemical examination of the blood or spinal fluid can be extremely helpful. The blood or spinal fluid sugar is very low in insulin shock deaths and tremendously elevated in diabetic coma. The Non-Protein-Nitrogen and Blood-Urea-Nitrogen are elevated in renal disease

with uremia. Fungi of various types may be isolated from the lungs or from the spinal fluid. In cases of Cryptococcosis the spinal fluid sediment stained with India ink will demonstrate beautifully the large capsule surrounding the Cryptococcus neoformans (Torula histolytica). Sections of brain and spinal cord can be frozen and sent to larger centers for analysis in the encephalitides and other viral diseases of the central nervous system. Bone marrow examination at autopsy can verify suspected cases of leukemia and metastatic bone disease and has been helpful in many other hematologic disorders.

In the medicolegal autopsy the various laboratory tests are extremely important. Blood stains on the victim's clothing or body can be identified as to type and differentiated from the victim's blood. In this manner the blood may be proven to be that of the assailant rather than the victim.

In many cases of human incineration in building fires, analysis of the blood or bones for carbon monoxide may prove the victim was deceased before the fire began. The fire may have been an attempt to conceal a murder.

In toxicological examinations for poisons such as barbiturates, heavy metals, etc., the following specimens should be saved:

One half of the brain
One kidney
A large fragment of liver
Gastric contents
Blood
Urine
Bone fragments

The specimens should be placed in large clean jars and sealed with no preservative added. They are then refrigerated in preparation for shipping to a central toxicological laboratory. Many of the simple tests such as for barbiturates may be accomplished by the technologist in his or her own laboratory.

The preparation of the tissue sections from the specimens removed at autopsy is similar to the preparation of slides from surgical material. Sections must be made from each of the carefully selected specimens taken at autopsy. Hematoxylin and eosin stains are adequate for most tissues but occasionally special stains must be used to best depict the pathological process.

The autopsy room is an important division of every pathology department and in the smaller hospitals the technologist must consider it as within his or her domain. This added burden should be accomplished with a sense of pride and responsibility as the technologist and pathologist work hand in hand to provide the best of pathology service for their patients, hospital and community.

Acknowledgment

I wish to express my appreciation to Dr. William G. Richards, Pathologist, for his advice and constructive criticism.

BACTERIOLOGIC DIAGNOSIS IN THE SMALL LABORATORY*

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The scope of bacteriologic work which can be satisfactorily undertaken by a small laboratory, with an adequately trained technologist and a limited supply of equipment, is considerably larger than is generally recognized. Numerous workers in small laboratories and clinics make no attempt to do determinative bacteriologic procedures, feeling that the materials and amount of work necessary for a service of this type are prohibitive. Most student technologists receive their training in large institutions and may be at a loss as to which techniques can be employed to advantage for a limited service. With these problems in mind, the author in training students, demonstrated and required students to work with certain cultural procedures using the materials which would be available in such circumstances. In the course of these studies, considerable experience in these techniques was acquired and it became apparent that the schema developed would be practical in a wide variety of post-training situations. Herein will be presented an outline of such a schema for bacteriologic diagnosis requiring only limited equipment and materials and a minimum time expenditure on the part of the technologist. The outline used allows the worker to terminate his presumptive identification at any one of several stages that prevailing conditions in his laboratory may dictate.

Since a laboratory may be defined as "small" in terms of equipment and space available, number of technologists, number of cultures requested and total volume of all types of laboratory work, the individual technologist must determine the practicability of each procedure in his particular situation. For example, in some cases it might be desirable not to provide for certain types of cultures rarely requested, as those for acid fast organisms, the strict anaerobes, gonococci, or diphtheria. It was felt however that most of the procedures could be handled in the average size physician's office.

Equipment and Materials

The basic equipment, materials, culture media, stains and reagents for the procedures to be discussed are listed in Table I. It is assumed that the bacteriologic work will be done in conjunction with other routine laboratory procedures, so that certain equipment will be mutually available. Most laboratories or offices are equipped with a refrigerator, bunsen burner, centrifuge, and possibly an autoclave. In lieu of the autoclave, an ordinary pressure cooker may be used for the small operations to be discussed here. A staining rack is readily improvised. As a candle jar, any container which can be tightly closed, such as a coffee can, will serve adequately. Loops, needles, test tubes, and petri dishes must be obtained. Petri plate handling can be obviated by the use of sterile disposable plastic plates. This equipment, together with dehydrated or prepared media and stains, constitutes the requirements for the procedures to be discussed.

* Read before the 26th Annual Convention of ASMT, Milwaukee, Wisconsin, June, 1958. Third S.P.F. Award—Bacteriology 1958.

Culture Media

Most of the culture media necessary for presumptive diagnosis are obtainable in prepared form. All media listed in Table I, excepting GC, are available from the major supply companies in plates or tubes ready for inoculation. These may be stored for several months. Of course, availability of refrigerator space and demand for certain types of cultures would dictate varieties and total amount to be stocked. Many types of media are available in disposable plastic plates. There are also sources of various types of prepared tubed media, as well as media in tablet form to be dissolved in sterile distilled water to give the required broths. Prepared blood culture bottles containing broth and CO_2 are readily obtainable. Various kits for certain procedures, i.e., identification of enteric pathogens, are marketed. These contain sufficient media of the required varieties for the isolation and identification of organisms from a single clinical specimen.

TABLE I
Requirements for Bacteriologic Schema

Equipment and Materials	
Incubator	Bunsen burner
Refrigerator	Centrifuge
Stain rack	Candle jar
Loops, needles	Pressure cooker (autoclave)
Test tubes	Plastic petri plates
Petri dishes	Anaerobic plates
Culture Media	
Blood agar	Triple sugar iron (TSI)
Chocolate agar	GC medium
Eosin methylene blue agar	Lowenstein-Jensen
Phenylethyl alcohol agar	Loeffler's
Salmonella-Shigella agar	Urea
Trypticase soy broth	Motility
Anaerobic agar	Thioglycollate
Stains and Reagents	
Gentian violet	Kinyoun's stain
Iodine	Oxidase reagent
Carbonate buffer	Methylene blue
Optochin discs	Albert's stain
Acetone-alcohol	Trisodium phosphate
Saffranine	
Biologicals	
Whole blood	Salmonella-Shigella antisera
GC supplement	Antibiotic discs

In the event the technologist wishes to prepare media, most varieties can be prepared in conveniently large amounts, tubed, and stoppered tightly with rubber stoppers to prevent drying. Under these conditions media can be stored under refrigeration for several months. Agar tubes may be melted to pour fresh plates as needed. The preparation of media for fermentation determinations can be simplified. A sugar free indicator broth can be stocked and prepared 10 percent concentrations of the various sugars can be purchased. As the need arises a single tube of the desired sugar broths can be prepared from these stocks.

Spaur and Wynne (1951)[7] described a simple method for determination of fermentation reactions in which filter paper strips impregnated with various sugars are embedded in separate portions of the same petri dish containing indicator agar. An acid reaction can be noted around the strip containing the sugar fermented by the organism inoculated. Impregnated discs to be used in a similar technique are available from commercial sources. In the author's experience, these techniques have yielded results comparable to tube fermentation tests.

The basic agar media needed are: blood, chocolate blood, phenylethyl alcohol, eosin methylene blue (EMB), Salmonella-Shigella (SS), Triple Sugar Iron (TSI), urea, and motility. GC medium, Lowenstein-Jensen, and Loeffler's should be available for appropriate cases. Trypticase soy broth in tubes and in bottles containing CO_2 and thioglycollate broth will serve for most cases where a liquid medium is required or preferred.

Stains and Reagents

Stains desired are Gram's, Kinyoun's, Albert's, and methylene blue. Depending upon the time available, these may be easily prepared or commercial stain solutions may be purchased. Certain biologicals are to be desired, blood for incorporation in culture media, glutamine and yeast supplement for GC medium, and antisera for limited *Salmonella* and *Shigella* typing. Trisodium phosphate for concentration procedures should be available. Optochin and oxidase reagent (*p*-aminodimethylaniline monohydrochloride) are also useful.

Antibiotic sensitivity discs in a myriad of forms are available from various sources. Where time or storage problems are a factor, probably one of the varieties incorporating several individual antibiotics in a single unit would be preferable. These are available with several assortments of antibiotics or can be prepared as specified by the technologist to meet his particular needs. The medium concentration of the antibiotics will suffice for most work.

Preparation and Plating of Specimens

It is assumed that the technologist will not be responsible for the collection of the specimens. However, he should be informed that a culture is to be sent so that he may make necessary arrangements, i.e., pour fresh plates of media or bring the required media to the proper temperature for inoculation. In the case of small laboratories, where the technologist has multiple duties, special care must be taken that specimens be inoculated promptly after collection. In select cases, the physician may prefer to inoculate the specimen to plates at the patient's bedside.

Observation of Gram's stains of direct smears of various materials can be of great help, not only as a preliminary report for the physician, but also in guiding the technologist in further procedures. For example, throat smears may be of value when the following are noted: (1) organisms having the morphological characteristics of *Corynebacterium diphtheriae*, (2) spirochetes and fusiform bacilli of Vincent's angina, or (3) budding yeast cells in large numbers characteristic of thrush. Smears from various exudates can be examined for organisms which may be presumptively identified as micrococci, streptococci, *Neisseria*, or as gram negative or acid fast bacilli. Gram's stained smears can be prepared and observed even

in offices having no equipment for doing culture work, thus aiding the physician in administering treatment until culture reports may be obtained from a distant source.

THROAT SPUTUM, EAR, EYE—Cultures may be inoculated on blood agar and in trypticase soy broth. Where *Hemophilus* is likely as a pathogen, i.e., the eye, chocolate blood agar should be plated also.

URINE—Urine specimens are centrifuged about 10 minutes, the sediment stained and planted to trypticase soy broth and EMB.

FECES—Specimens should be planted as soon as possible after collection. In the case of liquid specimens, or if a rectal swab is used, direct plating will usually suffice. Otherwise, emulsification in broth is indicated. An enrichment broth, easily prepared, such as tetrathionate may be desired. SS agar is a good selective and differential medium. EMB or MacConkey's agar should also be used.

SPINAL FLUID—Spinal fluids should be handled soon after collection by centrifuging and planting the sediment on blood agar, chocolate blood agar, and in thioglycollate broth.

WOUNDS AND EXUDATES—Swabs from wounds or sediment from centrifuged exudates, such as pleural or peritoneal fluids, may be inoculated to blood agar and thioglycollate. If both gram positive cocci and gram negative rods are observed in the direct smear, plating to phenylethyl alcohol medium at this time may facilitate early isolation of the gram positive organisms.

BLOOD CULTURES—Collection of blood directly into liquid medium is desirable.[3] Pour plates may also be prepared by melting a tube of trypticase soy agar, cooling, and adding the patient's blood.

ANAEROBIC CULTURES—Thioglycollate broth and anaerobic plates are convenient for isolation of anaerobes suspected in clinical materials.

ACID FAST BACILLI—Specimens likely to be sterile with regard to other bacterial organisms, i.e., pleural fluids, urines, spinal fluids, may be checked for sterility and planted to Lowenstein-Jensen medium. For such specimens, concentration procedures other than centrifugation may be dispensed with. For contaminated or highly mucoid specimens, such as sputum, trisodium phosphate concentration is easily handled. This method is particularly useful in the busy laboratory in that neutralization is not required, whereas in the sodium hydroxide method neutralization must be accomplished within a crucial time period.

Identification Procedures

In the small laboratory, identification, in most cases, must be on a presumptive basis. This fact should be indicated in the report submitted to the physician so that he may interpret the correlation between culture report and clinical findings in the proper light. Where technical procedures are limited, frequent consultation with the attending physician is of considerable benefit. Knowing what pathogens are suspected, the technologist can concentrate his efforts on procedures most likely to be of value. The physician, on the other hand, is alerted to the limitations of the methods employed in the laboratory.

The plating procedures suggested permit the isolation of the greatest number of pathogens with relatively simple media. In preliminary ex-

aminations, Gram's stain of the broth cultures and of colonies from plates is most important to indicate further steps. The discussion of further methods for identification will be based upon the observed colony characteristics, Gram's stain reaction and microscopic morphology. It should be understood that the procedures listed (Table II) may not be the best possible, but are amenable to modification according to the experience or preference of the technologist concerned.

TABLE II
Identification Procedures

Organism	Minimum	Further
Streptococci Enterococci	Hemolysis of blood Growth in 0.1% methylene blue—NaCl broth	Lancefield typing
Diplococci	Demonstration of capsule, "green" hemolysis, optochin sensitivity	Bile solubility, fermentations, mouse inoculation
Micrococci	Hemolysis, pigmentation, coagulase	Mannitol, gelatin
Neisseria Veillonella	Oxidase, pigment Anaerobic growth	Fermentations
Enterobacteriaceae	TSI reactions, urea, motility, polyvalent antisera	Fermentations, group antisera, referral for complete identification
Hemophilus	Growth only on media containing blood, hemolysis, "satellite" phenomenon	Bordet Gengou medium for B. pertussis
Brucella	Isolation on trypticase soy agar, or draw blood into medium only	Dye inhibitions, H_2S, Referral
Corynebacterium	Morphology	Virulence testing *in vitro* or animal
Bacillus	Aerobic growth, Morphology	Animal inoculation for anthrax
Clostridium	Anaerobic growth of morphologically typical organisms	Confirmation and identification by referral

GRAM POSITIVE COCCI—The commonly encountered organisms of this group are the *Micrococcaceae, Diplococcus,* and *Streptococcus.* The members of the *Micrococcaceae* usually isolated, *Staphylococcus, Gaffyka, Sarcina,* can be identified as such by the microscopic morphology. Staphylococci should be further characterized as to albus or aureus species depending upon pigmentation and coagulase reaction. Hemolysis on blood agar may also be of importance.

The streptococci can be classified by the type of hemolysis produced as alpha streptococci, beta streptococci, or gamma streptococci. If growth occurs only under anaerobic conditions, this notation should be made. It may also be of importance to determine whether or not the streptococcus isolated belongs to the enterococcus group. Enterococci

may be distinguished by their ability to grow in 0.1 percent methylene blue-6.5 percent NaCl broth.[5,6]

Pneumococci usually present no problem except in their differentiation from "green" streptococci. This may be easily accomplished, in most cases, by the use of optochin discs,[2] which in the author's experience give more clear cut reactions than bile or desoxycholate solubility tests.

GRAM NEGATIVE COCCI—The clinical source of the specimen can indicate to a great extent the probable classification of these organisms. Isolation and definitive classification of *Neisseria* from the nasopharynx is usually a relatively fruitless procedure. In genital specimens, findings should be reported only as "gram negative cocci, oxidase test positive." The oxidase test is important in confirming the presence of *Neisseria* since gram positive cocci showing variable staining and certain gram negative diplobacilli might otherwise be confused.

Neisseria isolated from spinal fluid are most likely *Neisseria* intracellularis but should not be so reported without confirmation by fermentation reactions. In most instances, however, the occurrence of these organisms in spinal fluid, in addition to his clinical observations, will provide the physician with sufficient information for proper treatment.

Gram negative cocci observed in thioglycollate broth should be shown to be anaerobic through attempting aerobic cultivation. Absence of aerobic growth indicates *Viellonella* species.

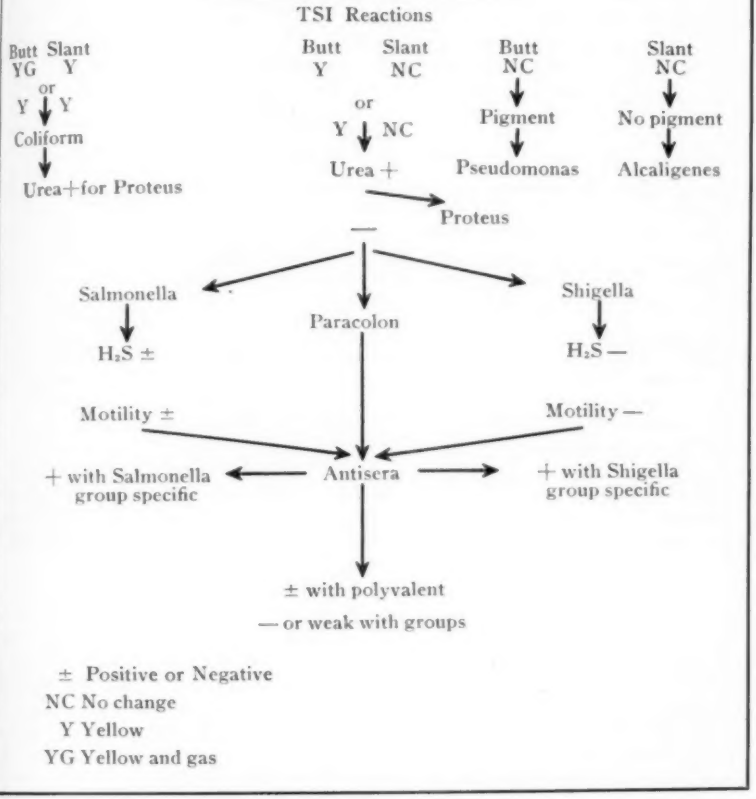
Gram Negative Bacilli

ENTEROBACTERIACEAE—Gram negative bacilli of the enteric group regardless of clinical source, may be identified by the same schema (Figure 1) once isolation has been accomplished. Differential media help in selection of certain groups of organisms. In any case, organisms to be investigated are carefully picked to TSI slants. From the results of 24 hour readings further tests are indicated. Coliforms may be classified as such at this time. Identification of *Pseudomonas* and *Alcaligenes* can also be made. Other reactions necessitate at least the planting of urea medium and motility determination. Proteus can then be either reported or eliminated from consideration. Depending upon reactions observed, *Salmonella* and/or *Shigella* antiserum may be employed. Fermentation and other biochemical tests are desirable in all cases where conditions permit, but it has been the author's experience that in cases where a choice must be made, typing techniques are much to be preferred in that they may be accomplished more easily and an earlier presumptive report can be rendered. Cultures may be sent to regional laboratories for complete identification of *Salmonella* or *Shigella*. It must be recognized that this schema may miss certain organisms of this group, i.e., some *Paracolobactrum* species.

HEMOGLOBINOPHILS—Isolation of these organisms will be of greatest importance in eye and spinal fluid specimens. Growth will first be observed on chocolate agar. Plates seeded with staphylococci will show the "satellite" phenomenon.[4] No growth will occur in media containing no blood. With this information, identification of *Hemophilus* for practical purposes, is accomplished. For the isolation of *Bordetella pertussis* special medium is needed. This, however, is seldom requested of the small laboratory and therefore is not included in the list of media.

Figure 1

Classification of Enterobacteriaceae



BRUCELLA—This group of organisms will usually be considered in this schema when a gram negative organism not identified by the foregoing procedures is encountered in a blood culture. The isolation of *Brucella* often requires a tedious and time consuming technique, therefore it is suggested that blood cultures from suspected cases be sent to an appropriate laboratory immediately following collection.

GRAM POSITIVE BACILLI—Aerobic gram positive bacilli are commonly nonpathogens either present in a lesion as saphrophytes or introduced into the culture through contamination. The recognized pathogen, *Bacillus anthracis*, can be suspected if typical colonial and microscopic morphology is present but should be confirmed, in all cases, by referral to a suitable laboratory for animal virulence studies.

If large Gram positive bacilli are observed in the thioglycollate broth and are shown to be anaerobic, an organism of the genus *Clostridium* is indicated. Complete identification is quite laborious, but a report of the presence of *Clostridium* species is of definite help to the physician as an adjunct to his clinical findings.

In cultures for diphtheria organisms, only a report as to the morphological and staining characteristics of the isolated organisms may be given. This is also true in the case of the acid fast bacilli. These organisms must be further characterized by virulence tests.

Sensitivity Testing

As an aid to therapy, antibiotic sensitivity studies are of utmost importance. These determinations are a necessity in the case of staphylococci and gram negative bacilli, but less so in the case of streptococci, pneumococci and gram positive bacilli whose sensitivities are more predictable. The enterococci are more resistant to various antibiotics and sensitivity determinations are required.

In some cases, notably those in which numerous organisms are seen in direct smears from the clinical materials, plates may be streaked from the swabs submitted and the sensitivity to antibiotics determined as soon as growth can be observed. For rapid results, it is advisable to do sensitivity testing on the mixed culture whenever possible. In this way, the test may be set up in many instances after a few hours preliminary incubation of the specimen in broth. A simple method for determination of sensitivity of anaerobic organisms has been described, in which discs are dropped into thioglycollate medium.[1] Results using this method compare favorably with those obtained by tube dilution.

Concluding Procedures

In the small laboratory, the occasion for referral of specimens to a larger laboratory for confirmation or complete identification will frequently arise. Consequently, it would be advisable to contact such a laboratory at the time the bacteriologic procedures are instituted. The laboratory to receive the referrals can then furnish information as to procedures for proper preservation, packing and mailing of specimens and in many cases furnish necessary materials. In most instances cultures should be submitted on slants. These tubes must be sealed with rubber stoppers or glass seal, packed in insulating material such as sawdust, glass wool or cotton and inserted in a double container, the innermost wall preferably of metal. The outside label should be marked "Notice—Biological Material—Avoid Extremes of Temperature." A suitable medium for referrals suggested in this paper would be blood agar. The referring laboratory should include with the specimen any pertinent information as to patient's history, site from which the specimen was obtained, the suspected organisms and the presumptive identification of the organism isolated.

The technologist, in employing at his laboratory the simple procedures discussed and resorting to referral in suitable cases, can furnish a relatively complete bacteriologic service with minimum effort and time expenditure.

Summary

A simple schema for bacterial isolation and identification with limited equipment and time expenditure has been outlined. The aim has been to encourage technologists in the physician's office, clinic, or small laboratory to undertake the performance of bacteriologic work. The procedures are amenable to modification according to preferences and experience of the technologist and the conditions prevailing in his particular laboratory.

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BOOK REVIEW

LABORATORY MEDICINE—HEMATOLOGY. By John B. Miale, M.D., Professor of Pathology, University of Miami School of Medicine, Director of Clinical Pathology, Jackson Memorial Hospital, Miami, Florida. The C. V. Mosby Company, St. Louis, Mo. 735 pages, 9 plates, 162 figures, 97 tables. $13.75.

The author of this excellent textbook on hematology has divided the subject matter into 12 chapters, each beginning with a list of the topics to be covered within the chapter in the same order and ending with an alphabetically listed and comprehensive bibliography. The bibliography is not annotated which makes for less distracting reading.

Of the plates, 5 are in color to serve as an atlas, each carrying 24 rather small pictures of individual blood and bone marrow cells and erythrocyte morphology. The nuclei are stained on the pink side rather than purple and the cytoplasmic granulation is indistinct. These plates are followed by an excellent table with cell descriptions that should prove helpful in learning the identifications of cells. The remaining 4 plates are in black and white showing cell structures by use of phase microscopy.

In Chapter 3 entitled BONE MARROW: MEDULLARY AND EXTRAMEDULLARY HEMOPOIESIS, there are 37 case histories presented with laboratory data, bone marrow differential counts and black and white pictures of bone marrow smears and bone marrow sections. The value of section material and the diagnostic features of each case are stressed. This chapter should prove invaluable to the residents in clinical pathology who as part of their training in hematology are learning to make diagnostic reports on blood and bone marrow findings.

(Continued on Page 342)

DIAGNOSTIC VIROLOGY: A NEW FIELD FOR THE MEDICAL TECHNOLOGIST*

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A comparatively short time ago, the layman was virtually unfamilar with the term virus, but now it seems to be the general consensus that this is the accepted term for any illness for which there is no other apparent etiology. This may be due partially to the fact that in recent years great advances have been made in the study of host-virus relationships, particularly the nutritional factors required for growth of these systems. The United States Public Health Service is largely responsible for much of this progress due to their grant awards to various laboratories, medical schools and hospitals throughout the country in order that diagnostic virus laboratories and research programs may be established. It was through such a grant that the Virus Laboratory at Charity Hospital in New Orleans was equipped and opened, August, 1956. The purpose of this paper is an attempt to interest the medical technologist in the field of diagnostic virology by describing briefly the methods and procedures used for the isolation and identification of viruses in our laboratory.

Diagnostic Procedures

There are essentially three basic approaches to the diagnosis of infections caused by viruses, these being (1) demonstration of the appearance, or of a rise in titer, of specific antibodies during the course of the illness, (2) isolation and identification of the causative agent and (3) examination of the infected tissues for pathologic alterations. Of these three approaches, antibody demonstration by complement fixation is the most profitable because a marked rise in antibody titer, when demonstrated in convalescent serum, definitely implicates the patient as having recently recovered from a given illness.[5] For this procedure, two samples of blood must be obtained. The first or acute phase serum should be collected sometime during the first three days from onset of symptoms. This is necessary for two reasons: (1) because antibodies develop early in the course of certain viral infections and may be present in low titer, and (2) because the patient may have antibodies in the blood before onset of the disease due to vaccination or a previous attack of the virus. The second or convalescent sample of blood should be drawn 21 days after onset of symptoms in order to detect a rise in antibody titer, if such occurs. Usually a sharp rise in titer, fourfold or greater, will be demonstrated if the findings are significant. The fundamental principles of the complement fixation test are the same whether viral or other antigens are employed. It is obvious that the richest source of viral antigen is likely to give the most satisfactory results in complement fixation procedures if the antigen is prepared and treated in such a way that it rules out the possibilities of anti-complementary factors or non-specific reactions. The following is a list of commercially prepared antigens and their sources: St. Louis, eastern and western equine encephalomyelitis antigens prepared from mouse brain; three influenza

* First SPF Bacteriology Award 1958. Read before the 26th Annual Convention of ASMT, Milwaukee, Wisconsin, June, 1958.

types, namely, the PR8 FM1 strains of Type A and the Lee Strain of Type B, all prepared from the allantoic or amniotic fluid of the chick embryo; psittacosis and mumps prepared from the yolk and amniotic fluid of the chick embryo; herpes simplex prepared from the chorio-allantoic membrane of the chick embryo; Q fever and rickettsial fever prepared from mouse brain or guinea pig spleen. There are still many antigens which cannot be obtained commercially; however, a few research virus laboratories have undertaken the preparation of some of these.

The demonstration of a fourfold rise in complement fixing antibody titer using the specific viral antigen along with isolation of the virus is generally considered positive proof of recent infection with the virus in question. A diagnosis based on virus isolation alone should be considered tentative since the patient may harbor one virus without ill effects, yet become infected with another unidentified viral agent. However, it may be justifiable to assume that the patient is infected with the virus isolated, particularly if the clinical symptoms of the disease agree with laboratory findings. Such evidence is considered presumptive. The most accurate way to determine the causative agent of a specific infection is to isolate the virus from material removed through biopsy or at autopsy.

Collection of Specimens for Virus Isolation

The usual types of material collected for viral studies include blood, spinal fluid, throat washings, stools, vesicle fluids, scrapings, biopsy tissue and post-mortem tissue, depending upon the nature of the illness. For instance, in upper respiratory diseases, throat garglings or nasopharyngeal swabs are taken; or in central nervous system involvement spinal fluid or stool specimens are collected. Although all viruses that have been isolated will grow only in living cells, they often exhibit a specificity for the type of cell. For example, the influenza viruses prefer certain tissues in the embryonated hen's egg, while adenoviruses grow well in HeLa cells. Consequently, one of our biggest problems is orienting those in authority in terms of appropriate material to be sent to the laboratory, with an indication as to the type of virus suspected. This, of course, is not always possible, but, for the most part, information gained from the clinical picture or epidemiological history would facilitate, to some extent, the types of living systems required for virus isolation procedures. For the successful recovery of a virus it is important that specimens be collected and handled properly.[2] Material must be collected as early as possible during the acute stage of the illness since the agent, in most cases, tends to disappear rapidly, once the disease is in progress. Sterile precautions should be observed in the collection of all materials, not only to avoid contamination, but also to protect the worker. Antibiotics must be added in sufficient quantity to stools, throat washings and other samples in order to remove contaminating micro-organisms. We have found that in the majority of cases 1000 units of penicillin and mycostatin and 1000 ug. of streptomycin are sufficient to inhibit bacterial and mycological growth. If inoculation of the material is not possible at once, the specimen should be kept frozen at —20°C. for preservation of the virus. Many viruses will remain viable for years if stored in sealed ampules in a CO_2 chest at —40° to —70°C.

Techniques Commonly Used for Virus Isolations

For the isolation and identification of viruses we use three differen culture media; namely, the embryonated hen's egg, two types of tissue culture (which are HeLa cells and monkey kidney cells), and the Swiss mouse. The four most common techniques for inoculating the hen's egg are as follows: the chorio-allantoic membrane, the allantoic sac, the amniotic sac, and the yolk sac.[1] It might be well to mention that other parts of the egg are used for inoculation purposes also, such as the different organs and blood vessels of the embryo, but we find that the four areas mentioned suffice our needs at present. For these four techniques before inoculation, it is important that the eggs be candled to determine fertility as well as the position of the embryo. The first inoculating technique mentioned involves the chorio-allantoic membrane which is found directly beneath the shell membrane and encloses the allantoic sac. This sac in turn contains allantoic fluid in which is suspended the yolk and the embryo within the amniotic sac. Eggs which have been incubated 12-14 days are most suitable for chorio-allantoic inoculation. To inoculate the chorio-allantoic membrane, one must first cut with a dental drill a segment 1-2 cm. sq. in the shell and gently lift the shell from the underlying tissues using a half spear point dissecting needle. By puncturing the shell membrane enclosing the air sac at the base of the egg, the air is released equalizing the pressure, and a greater space is created for the egg's contents. The chorio-allantoic membrane then drops away from the shell and is ready for inoculation. This is accomplished by holding the tissue to be inoculated with sterile forceps and gently rubbing it over the membrane, or if the inoculum is in suspension, 0.2 ml. is dropped onto the membrane by means of a syringe or pipette. Forty-eight hours are then allowed for growth of the virus. If a virus is present, there will be a generalized thickening of the membrane over the inoculated area and pock marks may be visible. If pock marks are observed the membrane is then harvested. The infected area is removed with sterile forceps and scissors and allowed to freeze for one to two hours. With a sterile pestle, the tissue is ground into a paste and suspended in tryptose phosphate broth. After slow centrifugation at 1000 RPM for five minutes, the material is then ready for the next procedure which may be a second passage on the chorio-allantois or a neutralization test. To identify the virus, a neutralization test is performed which involves the mixing of a specific antiserum of known titer with an equal volume of diluted material containing the agent. This is then inoculated on the chorio-allantoic membrane, and if no growth appears after the required length of time, the antiserum has apparently neutralized the virus and positive identification may be reported. Herpes simplex virus, which may be isolated from what is commonly called a fever blister or cold sore, grows well on this membrane as do the small pox and related viruses which are usually recovered from vesicle fluids or scrapings of the vesicles.

The next technique involves the inoculation of the allantoic sac. In this procedure, frequently, 10-12 day old embryos are used. A slit 0.5 cm. in length is made with a dental drill in the egg shell, and by employing a 1 inch, 25 gauge needle, 0.2 ml. of bacteria-free inoculum is

injected directly into the allantoic cavity. After 36-48 hours' incubation period, the allantoic fluid is harvested by snipping the shell away from the base of the egg and gently folding the underlying membranes back over the sides. The fluid may then be removed by means of a capillary pipette. Since most viruses, such as influenza A and B, which grow in the allantoic cavity are capable of agglutinating chicken red cells, a hemagglutination test is performed for demonstration of the virus which, if present, is then identified by the hemagglutination-inhibition technique. This procedure requires specific antiserum which is mixed with an equal volume of infected fluid to which chicken red blood cells are added. If agglutination is inhibited for one hour, the test may be considered positive for the virus corresponding with the antiserum.

In applying the third technique cited, namely, inoculation of the amniotic cavity, a segment must be cut from the shell as previously described, and using a 1½ inch, 22 gauge needle, 0.2 ml. of bacteria-free inoculum is introduced into the cavity. Mumps virus, influenza C and the Asiatic strain of type A influenza viruses grow best in this part of the egg. Age of the embryos used depends on the virus suspected. Harvesting of amniotic fluid is performed in much the same manner as described for that of allantoic fluid, although some difficulty may be encountered since amniotic fluid volume is so small. Hemagglutination inhibition is the confirming test for positive identification. It might be well to reiterate at this point that viruses grow only in living cells, and do not actually grow in the allantoic and amniotic fluids, but in the surrounding endodermal cell layer. As multiplication of the virus particles takes place, the cells erupt and the particles are released into the fluids. Some viruses, such as herpes simplex, remain intracellular and the entire cell is released into the fluid.

The fourth technique, which is that of the yolk sac, is the simplest of all the egg inoculating procedures. Seven to nine day embryos are usually employed, and by means of a 2 inch, 22 gauge needle, 0.25 ml. of inoculum is introduced into the yolk sac via the air sac. Seventy-two hours is the incubation period required for multiplication of the virus. The eggs are then candled and those which contain dead embryos are prepared for harvesting. Psittacosis virus grows well in the yolk sac, yet there are other viruses which may be inoculated by this route, but are found, after incubation, to be in the allantoic or amniotic sac. The methods previously described are used for identification.

To isolate adenovirus, herpes simplex, polio and polio-like viruses such as Coxsackie and ECHO the tissue culture technique is employed.[4] A standard procedure for this technique has not been recommended because methods and procedures are constantly being changed following improvements. The types of tissue and other materials which are available or easily accessible often govern the method followed in different laboratories. It has been found that cells of tissue removed from a living host when isolated and placed in a nutrient solution will not only continue to metabolize but will continue to grow.[3] In our laboratory we use living cells of two different types: HeLa and monkey kidney cells. HeLa cells which are epithelial cells of carcinomatous origin were removed from the cervix of a patient by the name of Helen Lane—hence the term HeLa. Since their removal in February, 1951, these cells have

been propagated extensively, and because of their rapid growth and susceptibility to many viruses, especially polio and the adenoviruses, they are now used in many laboratories throughout the country.

The multiplication of these cells is accomplished by allowing them to grow for a ten day period at 36°C. on the inside surface of 150 ml. culture bottles. A nutrient growth medium is kept on the cells containing the following: a balanced salt solution with phenol red indicator, 10% human serum, glutamine, amino acids, vitamins, glucose, penicillin and streptomycin and enough $NaHCO_3$ to adjust the pH to 7.2-7.4. Since the pH and many other factors influence cell growth and virus multiplication, water which has been triple distilled through glass must be used in preparing the different solutions because metal tends to give off ions that are toxic. Also, the glassware used in the tissue culture procedures must be chemically as well as bacteriologically clean. The cells will have multiplied sufficiently after 8-10 days to be removed and either placed in other bottles for further growth or put into tubes in preparation for virus inoculation. There are several acceptible methods for removing cells from the wall of a culture bottle. We have found that trypsinization, 0.25% trypsin in Hank's solution, is a very satisfactory procedure. After removal from the culture bottles, the cells are suspended in 10 ml. of growth medium, counted in a hemacytometer and diluted sufficiently with growth medium so that there are 1×10^5 cells per ml. Approximately 5×10^4 cells per ml. are placed in pyrex culture tubes, which are held stationary in racks slightly inclined at a 5° angle. These cells are then incubated for two days at 36°C. during which time they will attach to the walls of the tubes, multiply and eventually form a sheet or network over the surface. Since the human serum present in the medium contains antibodies, it must be removed before the cells are ready for inoculation. This is achieved by removing the growth medium, washing the cells with balanced salt solution containing 5% rabbit serum, and replacing this solution with 1 ml. of maintenance medium. The latter is essentially the same as that used for growth except 10% rabbit serum is substituted for human serum. The tubes are now ready for inoculation. The monkey kidney cells are obtained already prepared for inoculation from a biological supply house since the preparation of these cells for tissue culture involves more time, working space and personnel than we have available. To inoculate monkey kidney cells or HeLa cells 0.2 ml. of inoculum is pipetted into each of as many tubes as used per specimen (four tubes are commonly employed). The tubes are incubated at 36°C. and the cells are observed microscopically daily for seven days or until they degenerate. This cytopathic effect is characterized by the cells becoming rounded and falling off the sides of the tubes. Cytopathogenicity may be due to viral infection, toxicity of the inoculum or bacterial contamination. An effect caused by bacterial contamination may be obvious, but to differentiate a viral attack from a toxic inoculum requires further study. After a cytopathic effect takes place, the remaining cells on the sides of the tubes are scraped down, the contents are pooled and four culture tubes are inoculated with this material. Any toxic substance present is diluted to such an extent after the second inoculation that it will not prove harmful to the cells. A cytopathic effect would then be indicative of

viral attack and should be followed by a neutralization test performed for identification of the virus. Antisera for the three types of polio and a few of the polio-like viruses are commercially available, but it must be emphasized that positive identification may not be possible since there are only a limited number of antisera other than polio, obtainable at present.

In recent years, embryonated hens' eggs and tissue cultures have to a great extent, replaced the use of animals as susceptible hosts for viral studies. The Swiss mouse is still used in many instances, especially for studies of neurotropic viruses. Three mice are commonly used for inoculation when these animals are three or four weeks old, but younger mice are more effective in some cases. Injections of .03 ml. are usually made intracerebrally, after first anesthetizing the mouse; however, inoculation by the subcutaneous, intramuscular or intraperitoneal route may be effected. If the material injected contains virus, the mice will die within 10-21 days. Following death, the brains are removed, frozen, ground into paste, suspended in a proper medium and reinoculated into three more mice. If death occurs after the second passage, the brains are harvested and sent to the Communicable Disease Center in Montgomery, Alabama, for identification. This is done for two reasons: (1) because there are not many antisera of the encephalitides groups available for neutralization tests in mice, (2) the encephalitis viruses are highly infectious and at the present time there is no vaccine available for protection of the investigator against many of these agents. Therefore, we try to handle highly infectious material as little as possible.

Observations

Viral diagnostic work is extremely costly and time consuming, and many of the tests are not completed until after death or complete recovery of the patient. For these reasons a diagnostic virus laboratory would not be feasible for the small hospital at present. However, it has been observed that virology is today where bacteriology was 50 years ago. With the closing of each year, the day rapidly approaches when a virus laboratory will become an integral part of many hospitals. This field can, therefore, offer a great challenge to the medical technologist interested in identifying the causes of many previously undefined infections.

Summary

The field of diagnostic virology is a new and challenging one. An attempt has been made to interest the medical technologist in this field by describing briefly the methods used in isolating and identifying viruses in the Virus Laboratory at Charity Hospital in New Orleans. These methods include the complement fixation technique, chick embryo and mouse inoculating technique and the procedure for tissue culture maintenance and inoculation.

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BOOK REVIEW (Continued from Page 335)

Chapter 12 on HEMOSTASIS AND BLOOD COAGULATION is another gem, for here one finds the most lucid and satisfactory explanation of the bleeding and coagulation mechanisms, the various factors that may interfere with normal coagulation and how to go about tracing the culprit involved. Giving the various factors Roman numerals should be adopted universally because of the confusion in terminology by different researchers in this field. The author places great emphasis on deficient vascular functions in bleeding disorders.

The newer concepts in hematology are broached upon to start us thinking in biochemical lines such as erythropoietin, humoral factors and the role of hormones in hemoglobin synthesis.

Hematological procedures are covered in a clear and concise manner in the appendix. The numbered step by step methods will appeal to medical technologists. They will be delighted by the inclusion of the more recent advances in the field. The Sanford method of performing the osmotic fragility should be omitted since the highest concentration of NaCl is only 0.50 per cent and the drop method is outmoded by current scientific methodology.

The index needs expansion for reference use. Many of the subtitles under a general heading deserve to be placed in the general index also.

The author's writing ability must be extolled. In clearly and beautifully expressed sentences he can convey his concepts to the reader better than in any textbook this reviewer has encountered. This book is a MUST for all in the field of hematology and should be in the library of every AMA Approved School of Medical Technology.

A quotation from the author's preface is in order: "This book on HEMATOLOGY and two others in preparation on CHEMICAL PATHOLOGY and MICROBIOLOGY emphasize the correlation between laboratory and clinical data. It ranges from basic mechanisms to the interpretation of data in diagnosis and therapy. Only tests which are ordered with discrimination, performed with skill, and interpreted with understanding yield significant data. With this in mind, the present and companion volumes are directed to medical students both in clinical pathology and in the transition to clinical medicine; to clinicians who must appreciate the value and limitations of laboratory data in ordering tests; to pathologists for reference and teaching; and to medical technologists, in whose integrity and skill physicians and patients place their trust."

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